

Cleavage sites of endoproteases on the a-chain of GPIb

FIG. 1

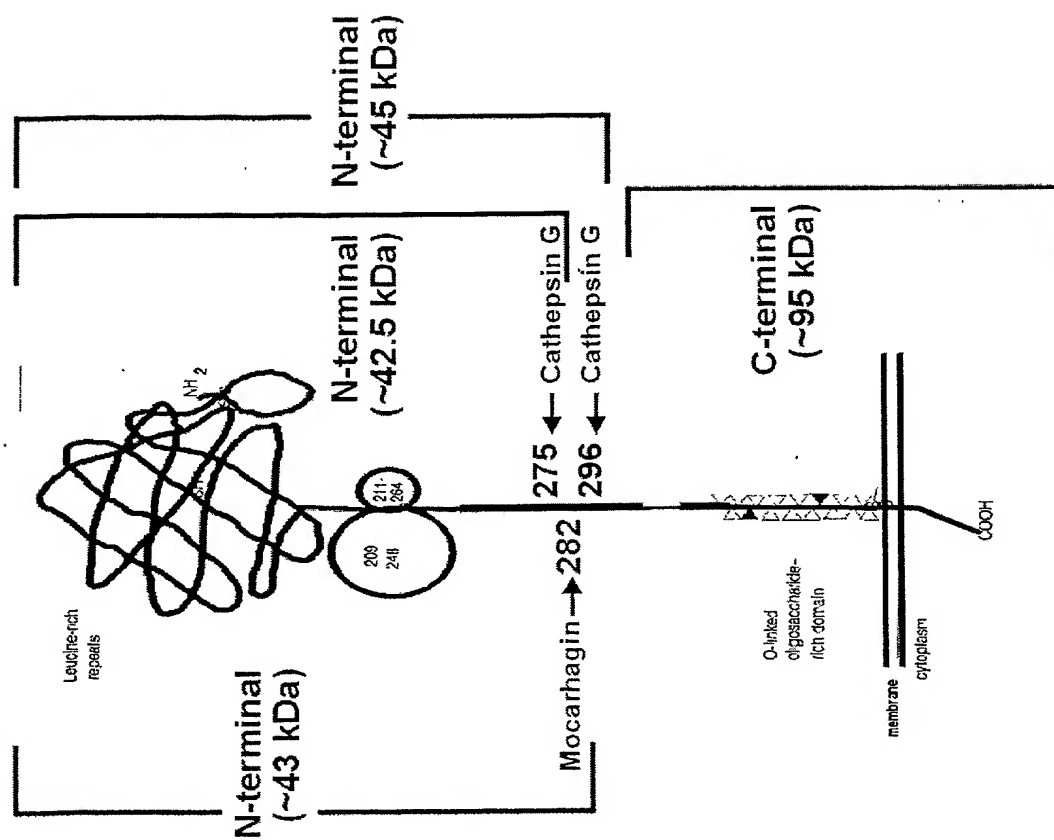


FIG. 2

Binding of Y1 and Y17 to platelets in reduced and non-reduced conditions

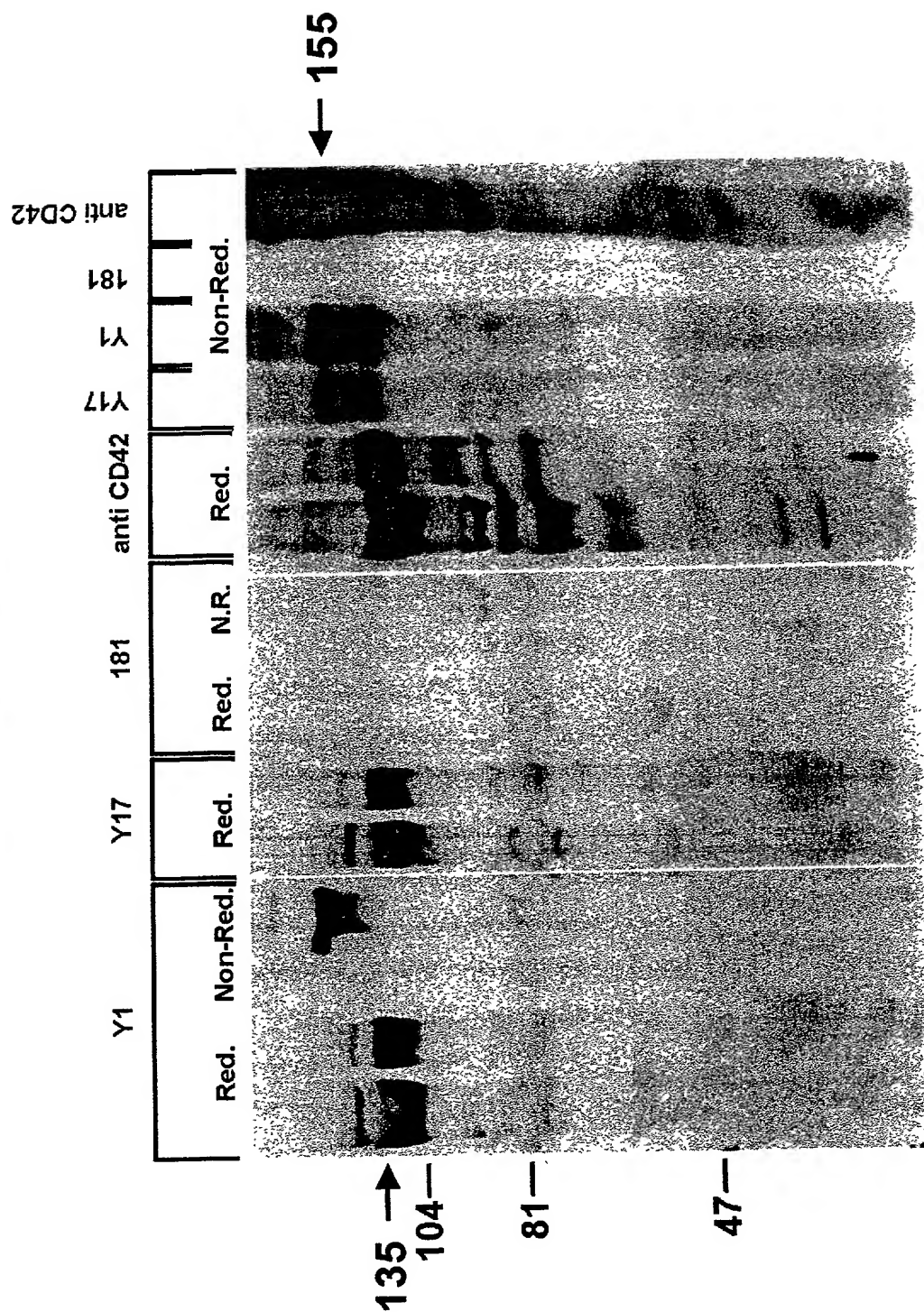


FIG. 3

Characterization of Optimal Determinants for Binding of Y1 to It's Ligands

	Platelets/GC	KG1/RP-HPLC #4
Rec: GP1b 1-340 GP1b 1-480	- -	
Glycanase: N N+O	+ +	+++ +++
Proteases: Mocarhagin O-Sialo Peptidase Ficin Trypsine Elastase	++ (~40kD) ++ (~40kD) - ++ (~40kD) ++ (~40kD)	- - - - ++
Sulfatase (Aryl)		-/+

Cleavage of platelets GPIb by O-Sialoglycoprotein abolishes binding of both Y1 and Y17

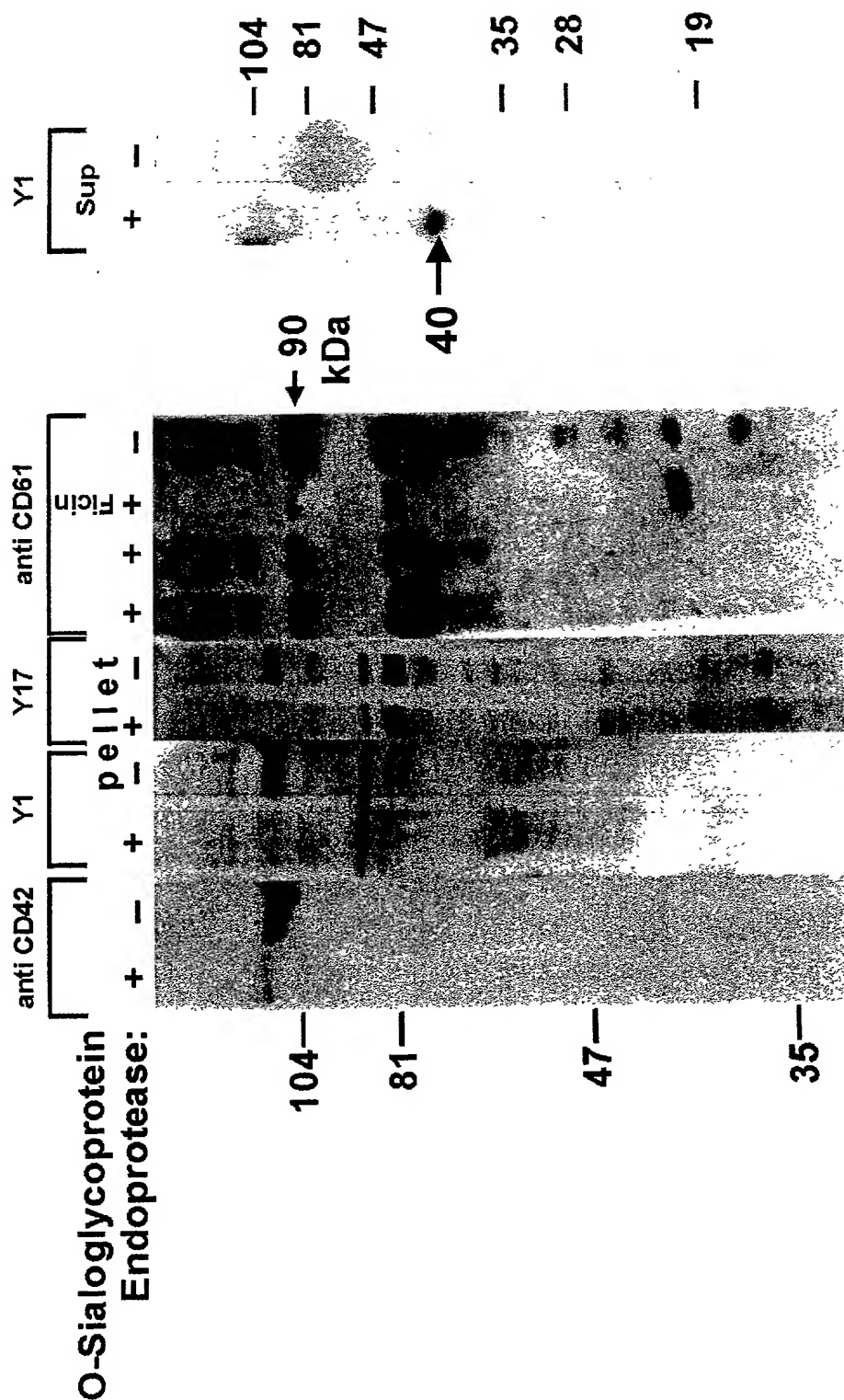


FIG. 4

Y1 and Y17 binds similar glycosylated fragments after cleavage by O-Sialoglycoprotein Endoprotease

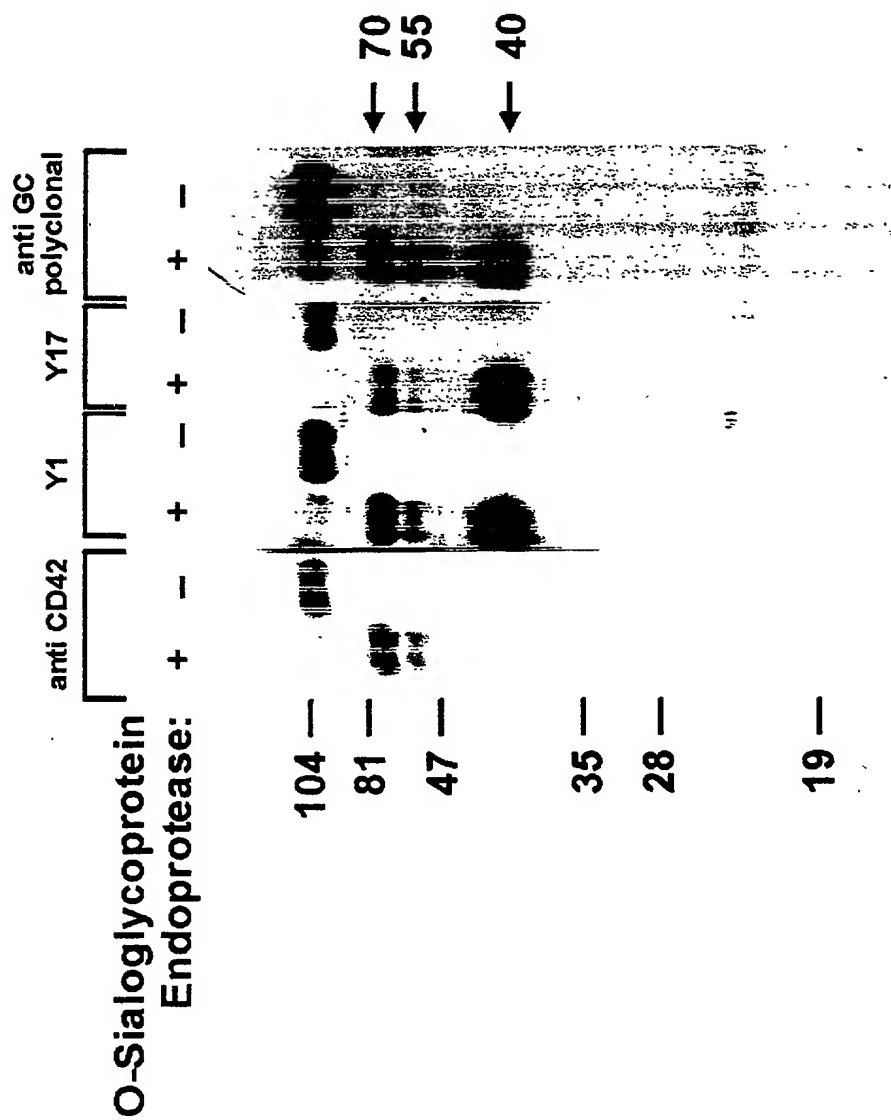
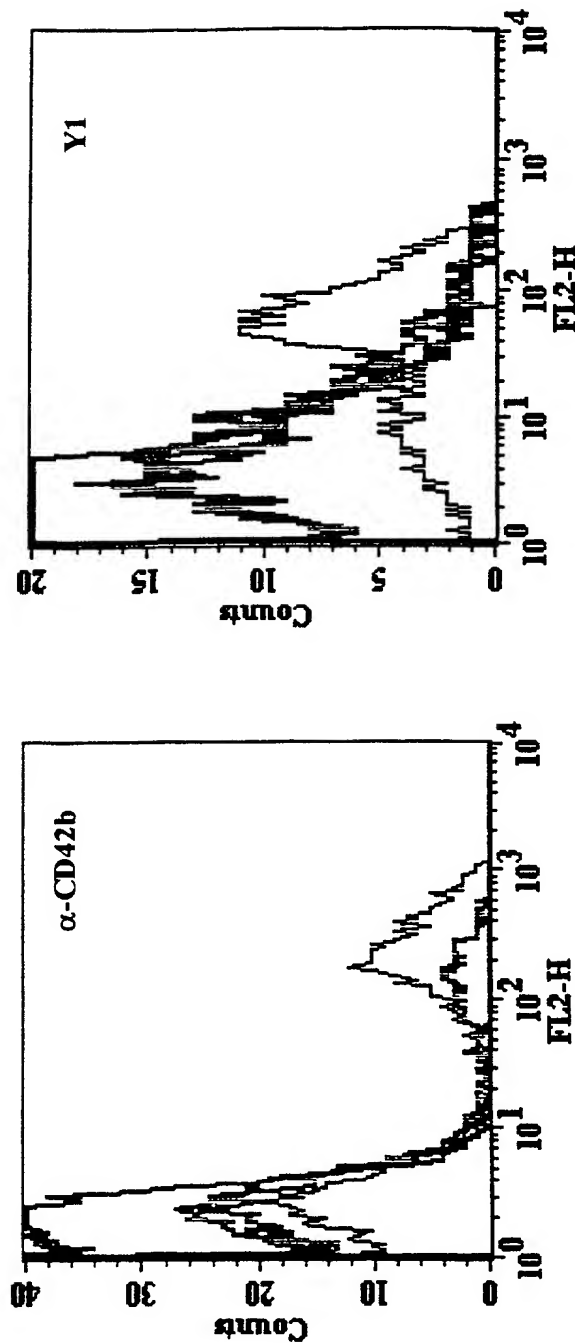


FIG. 5

Specific GPIb Proteolysis Abolishes Y1 Binding to Platelets



Key	Name	Parameter	G
	NON-TREATED PLATELETS		
---	O-SIALOGLYCOPROTEIN ENDO. (10 μg/ml)		
---	O-SIALOGLYCOPROTEIN ENDO. (50 μg/ml)		
---	FICIN (18 μg/ml)		

FIG. 6

Y1 binds N-terminal (His-1 - Glu 282) fragment of platelet GPIb after cleavage by mocarhagin

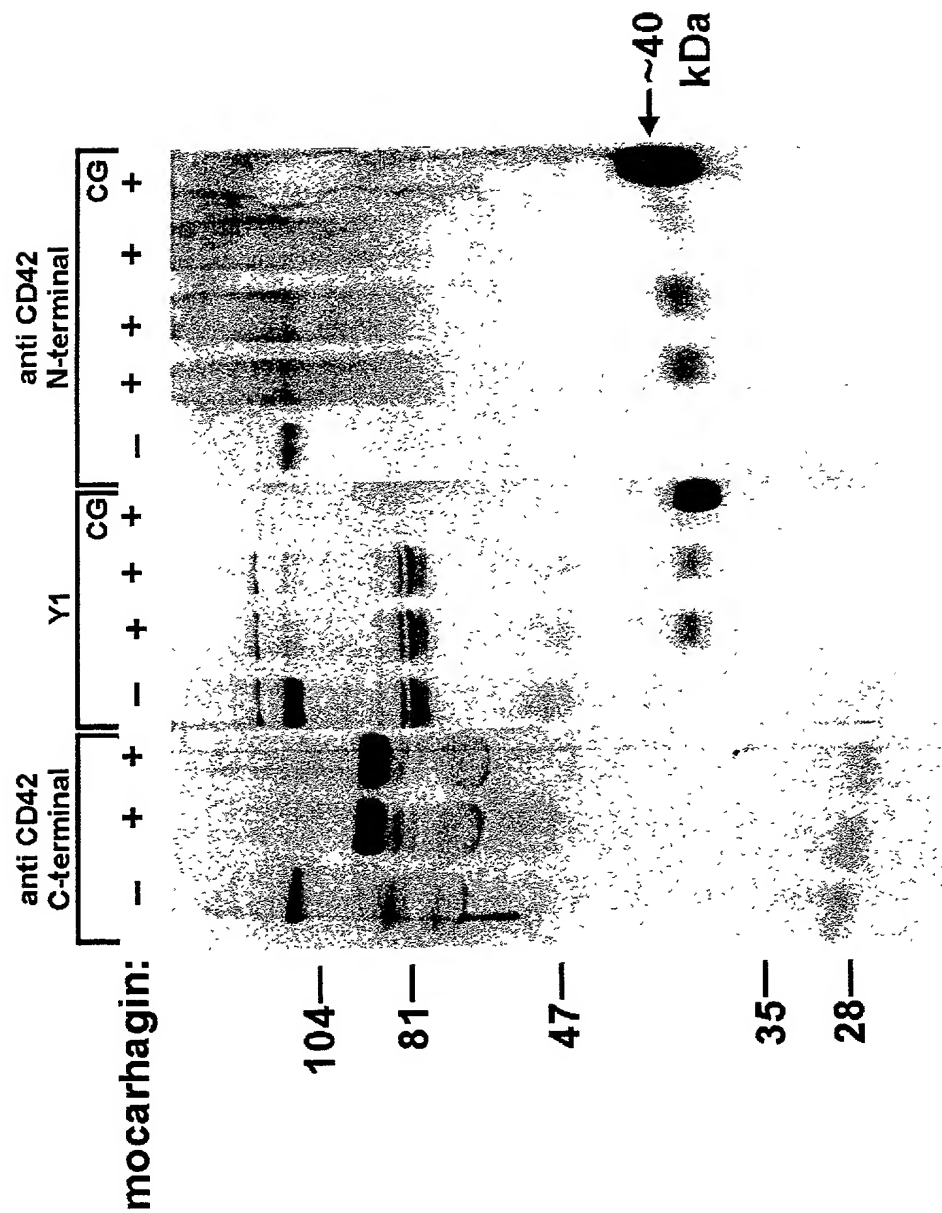


FIG. 8

Binding of Y1 and Y17 to glycolalycin after cleavage by mocarhagin

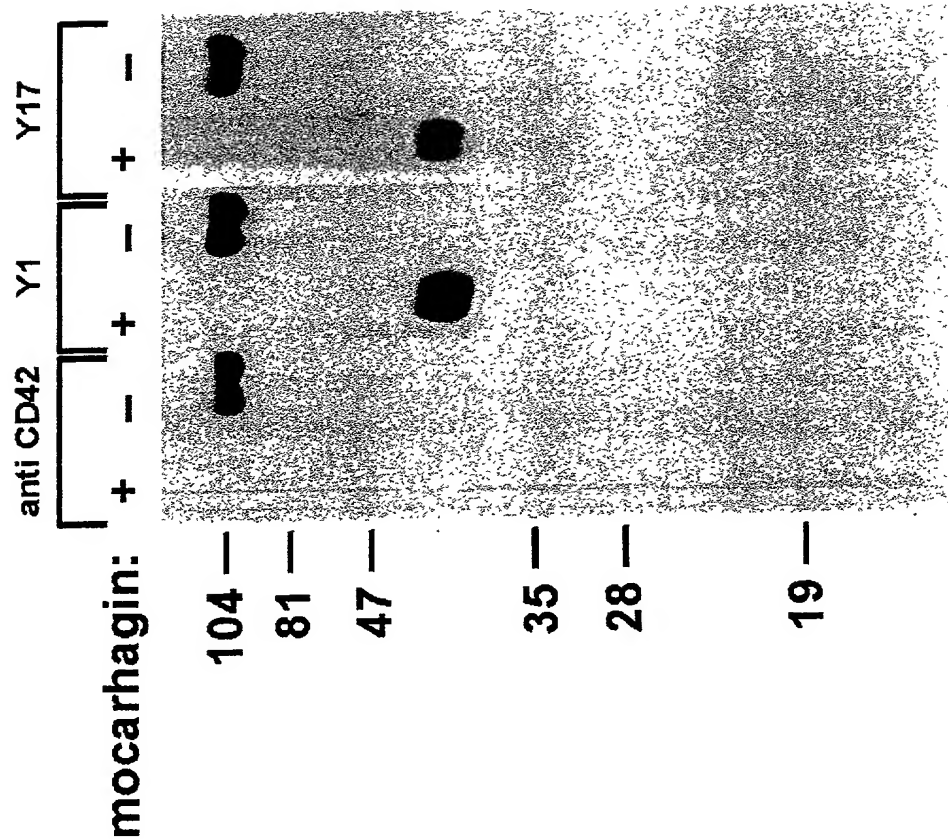
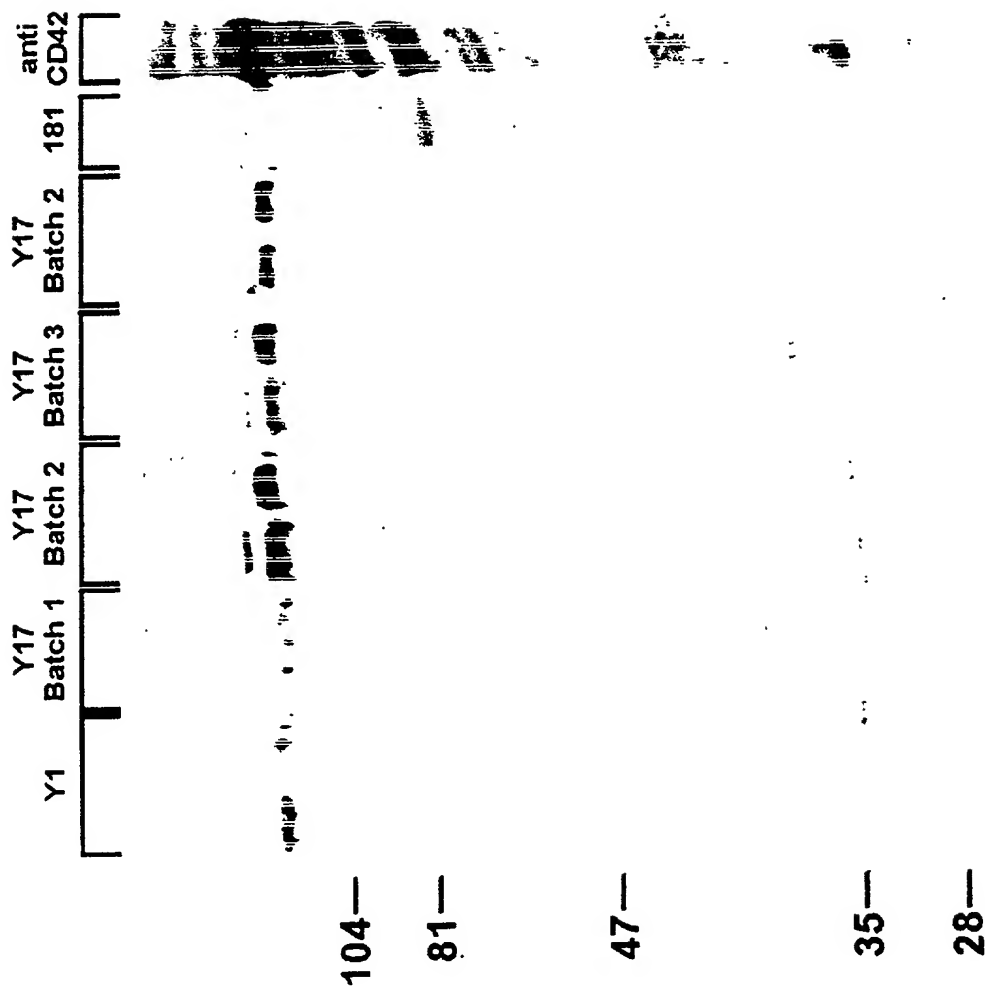


FIG. 9

Binding of Y1 and Y17 to platelets



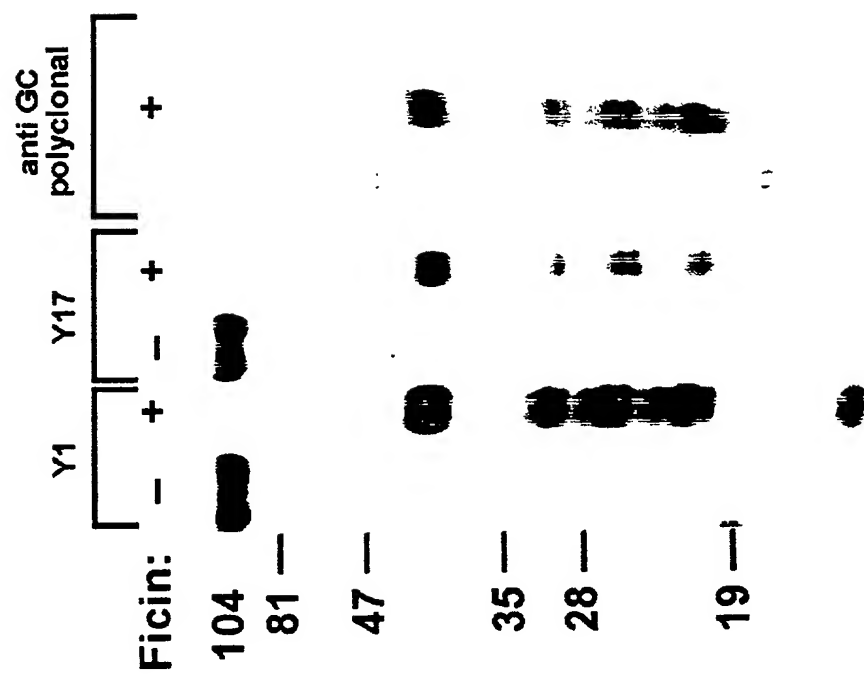
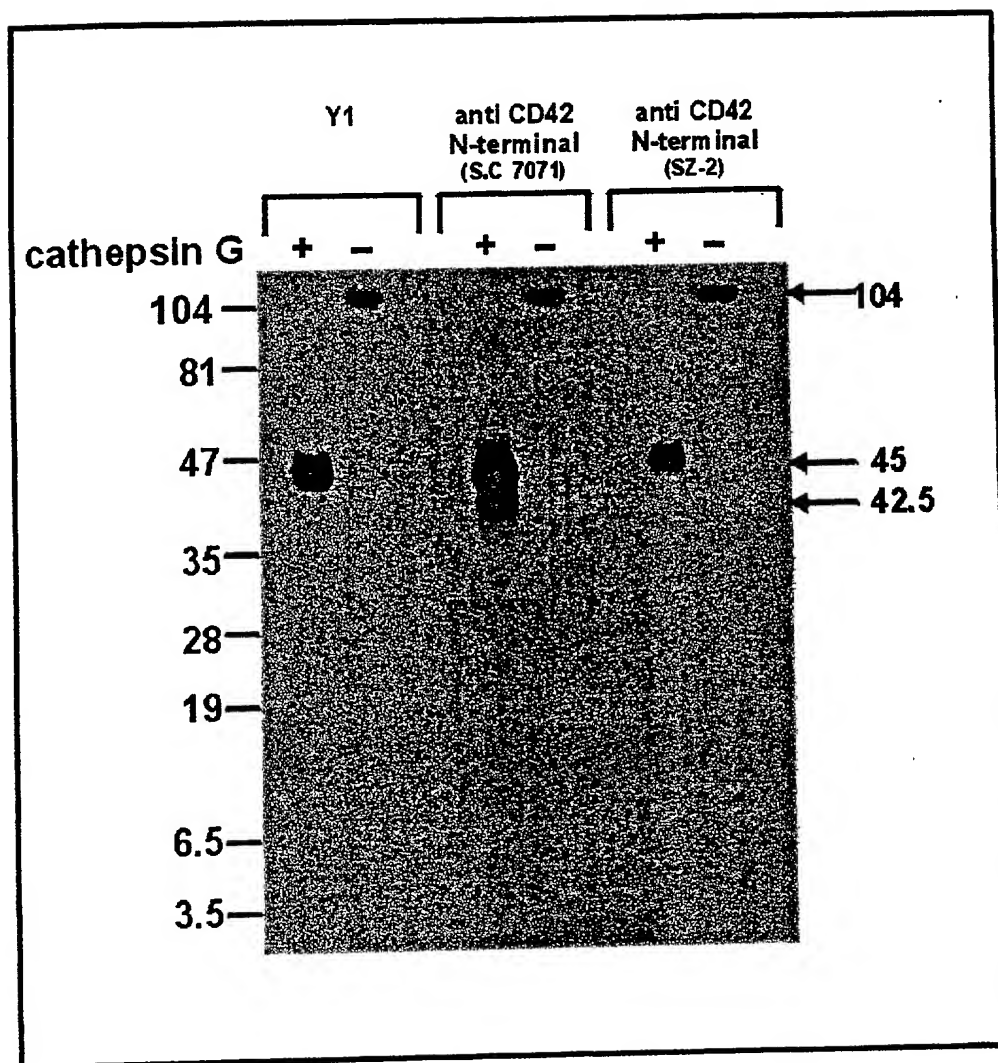
[illegible][illegible]

FIG. 11



Y1 and Y17 reacts with larger cathepsin G cleaved platelets GPIb fragment

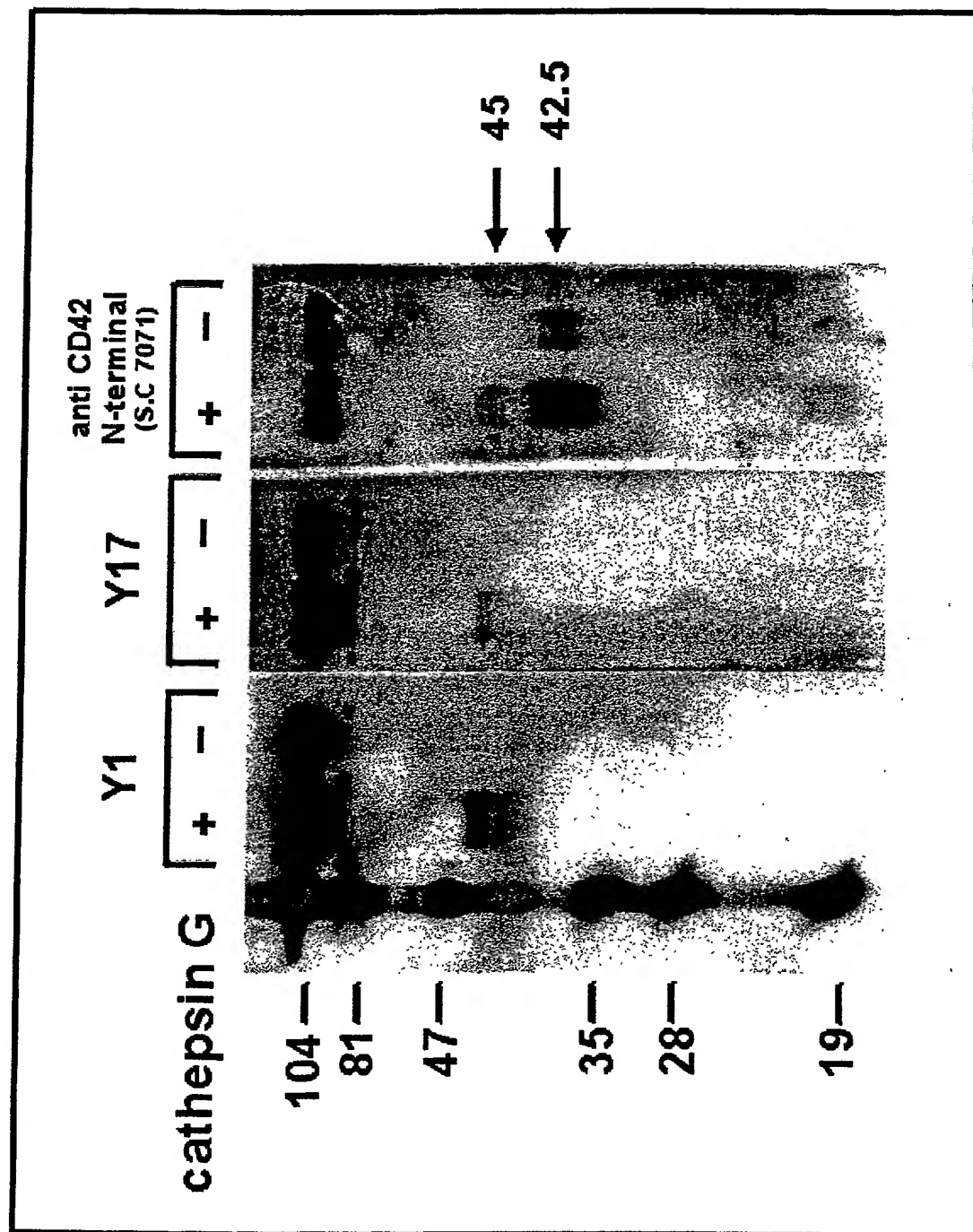
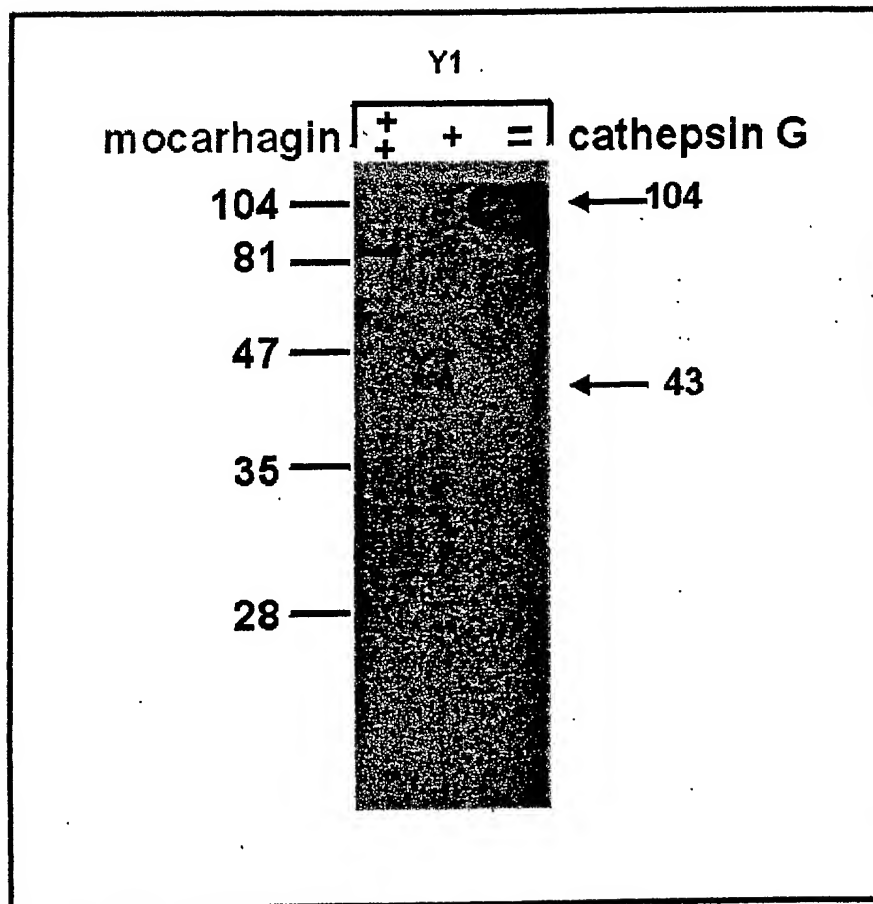


FIG. 12

FIG. 13



Cleavage of washed platelets by mocarhagin and cathepsin G

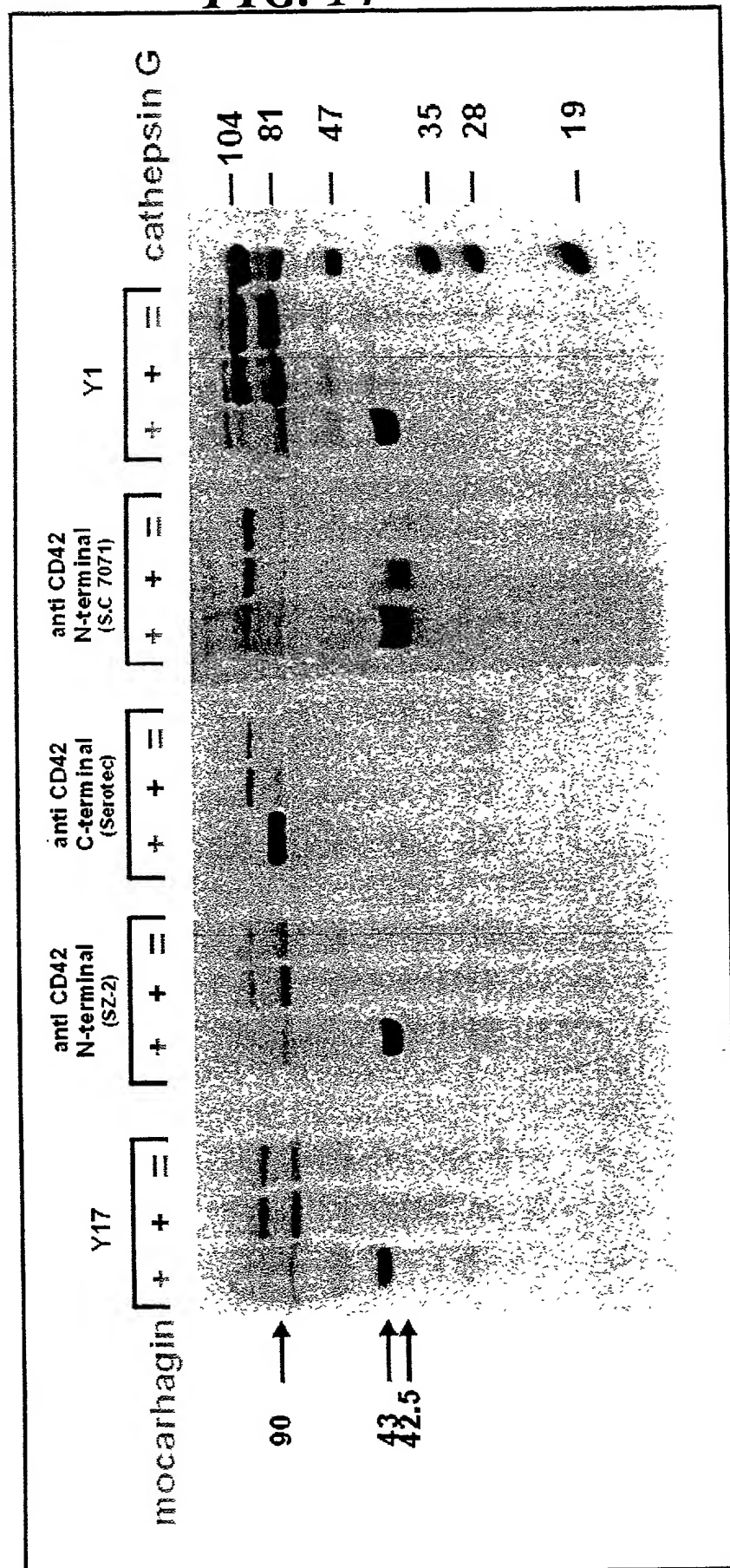


FIG. 15

Influence of Y1-scFv on platelets agglutination in washed platelets

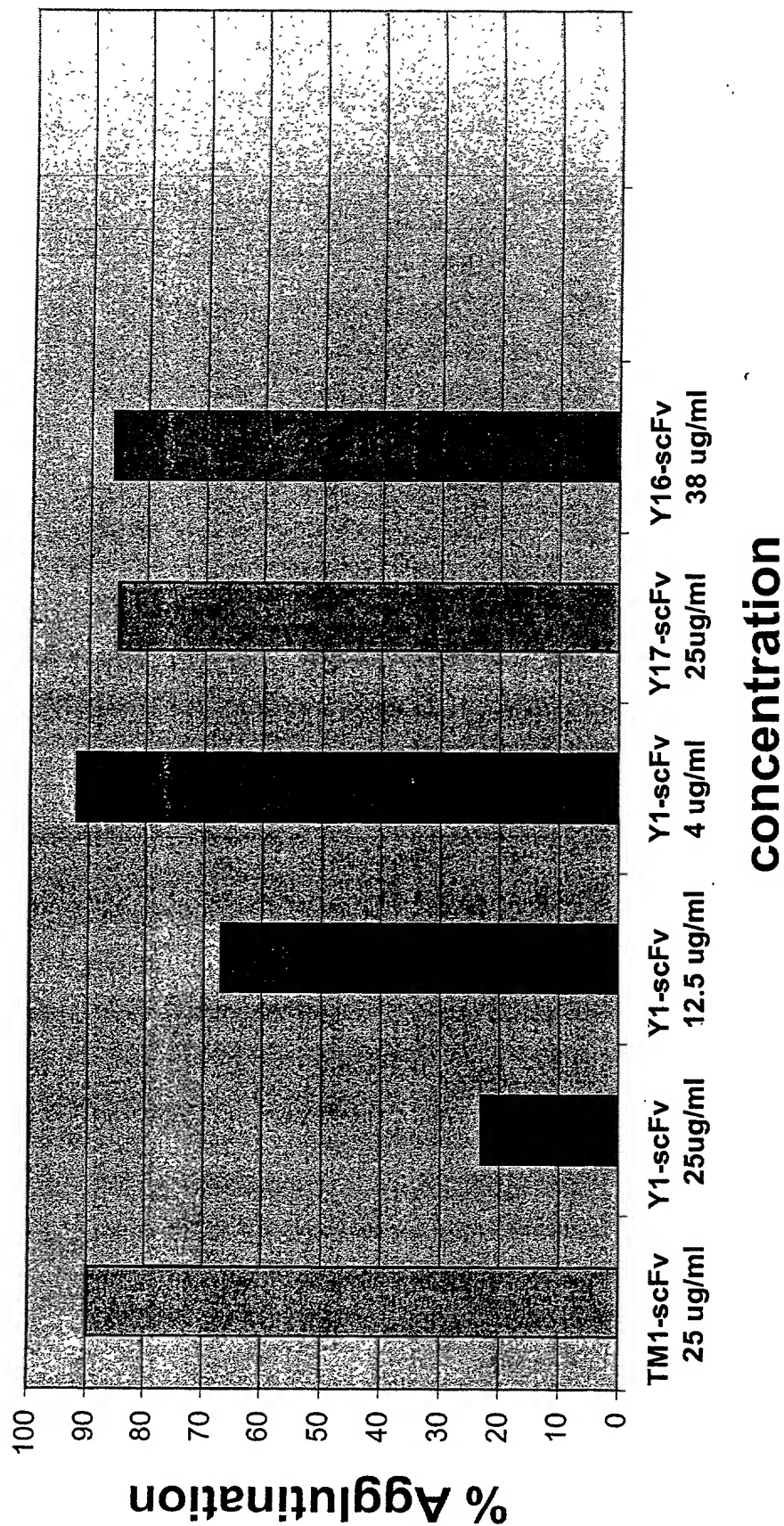


FIG. 16

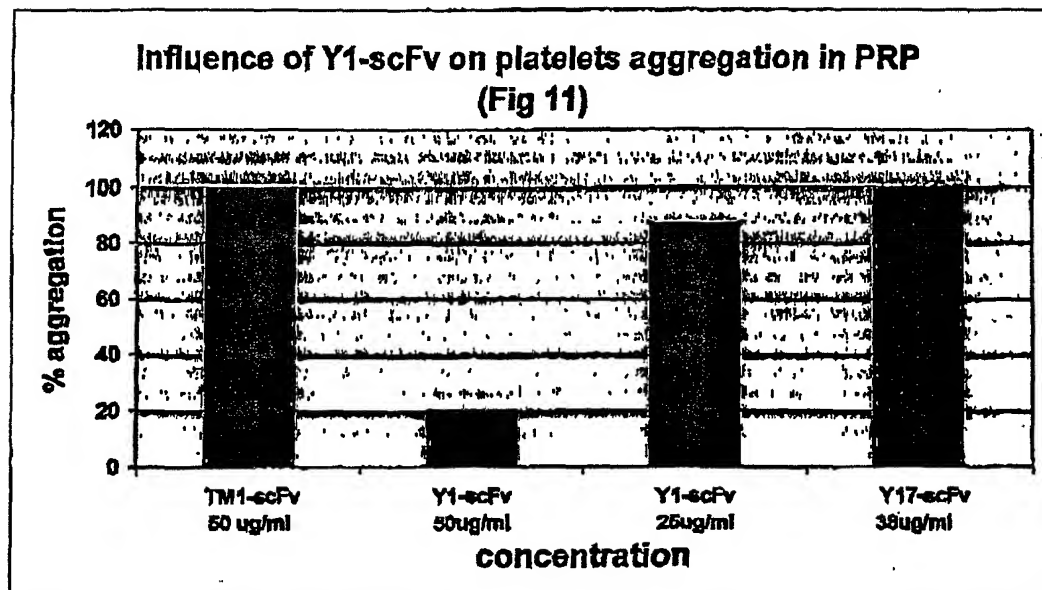


FIG. 17

Induction of platelet agglutination by Y1-IgG in washed platelets

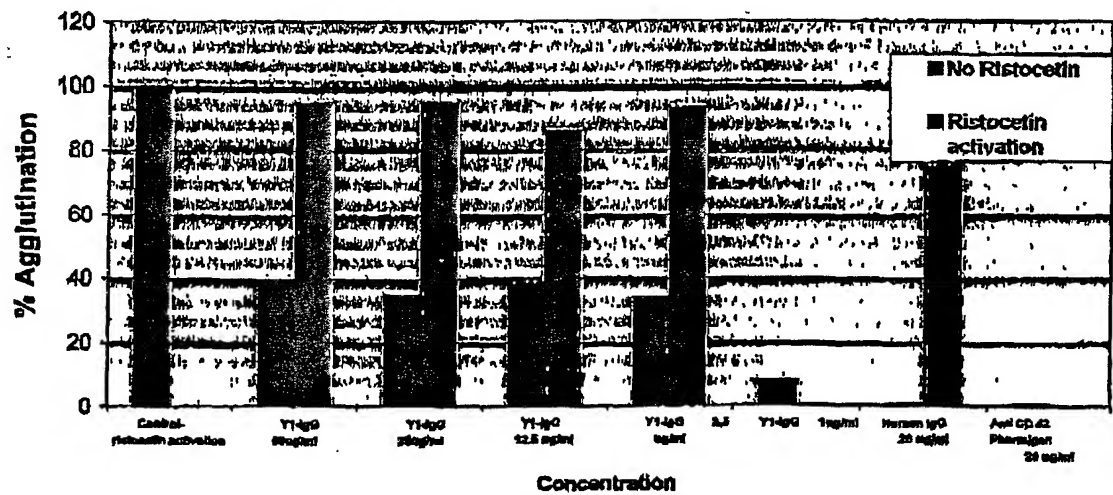


FIG. 18

Induction of platelet aggregation by Y1-IgG in PRP

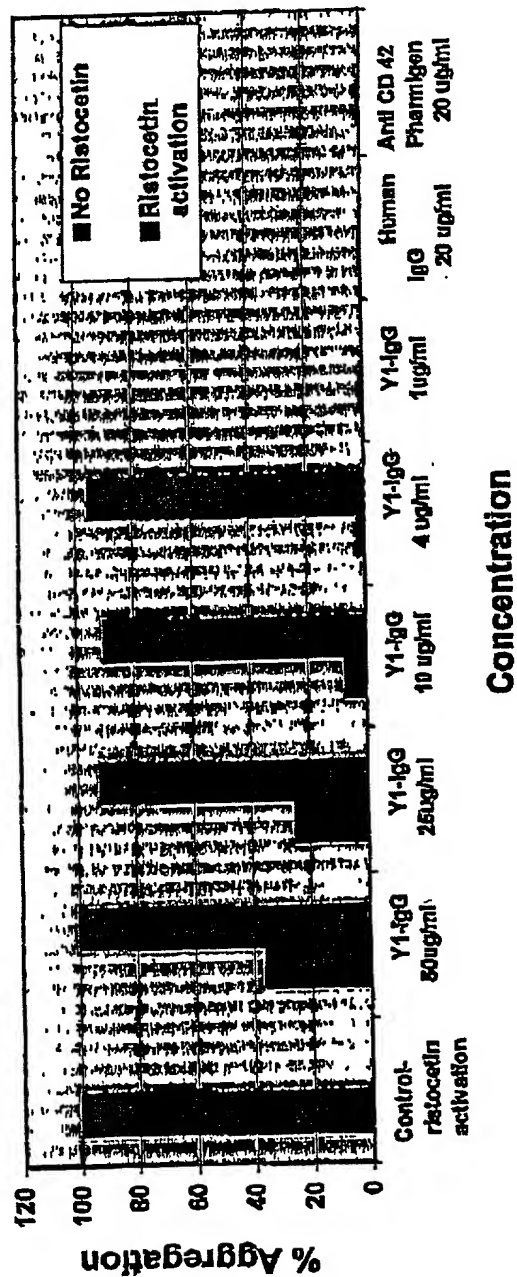


FIG. 19

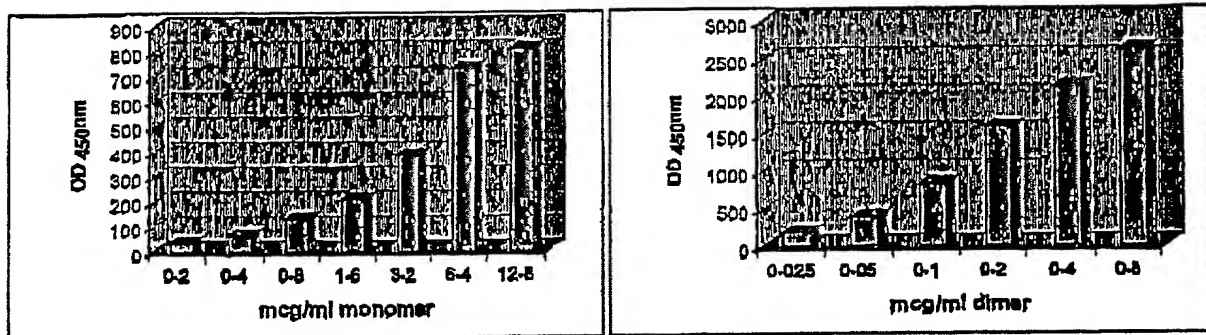


FIG. 20

**Specificity of Binding of Y1
and α -CD42 (N1-19)
to their Ligands**

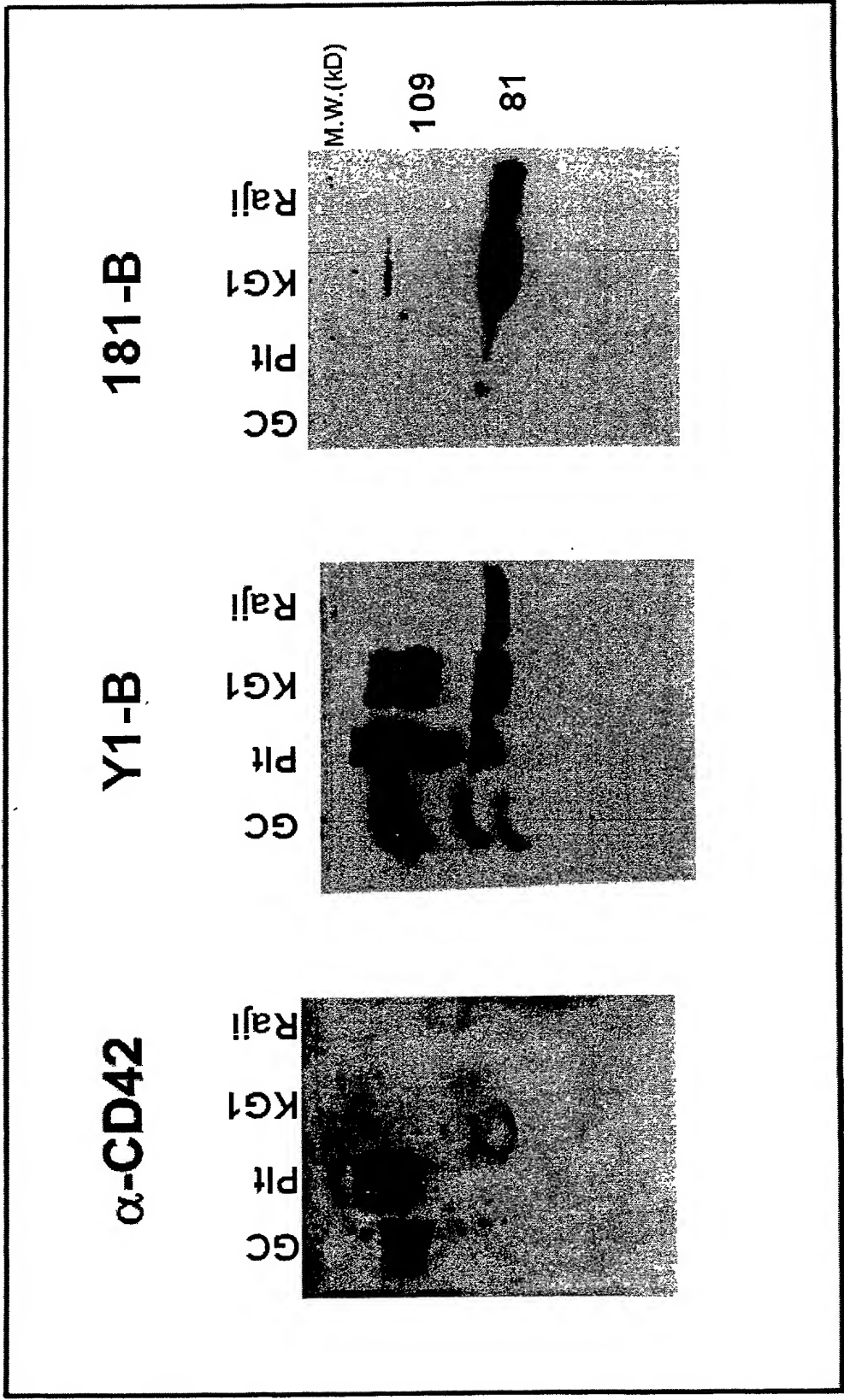
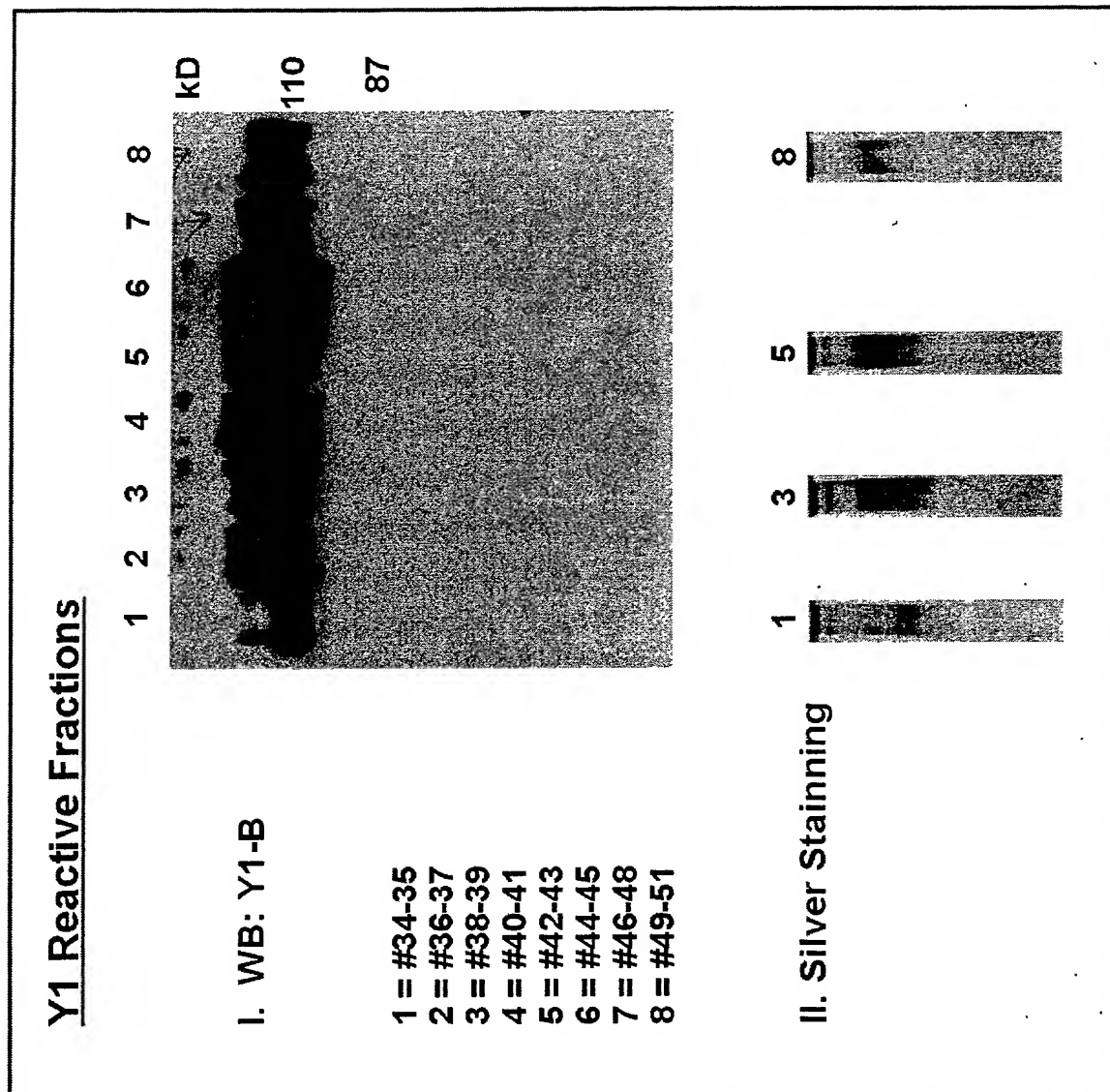


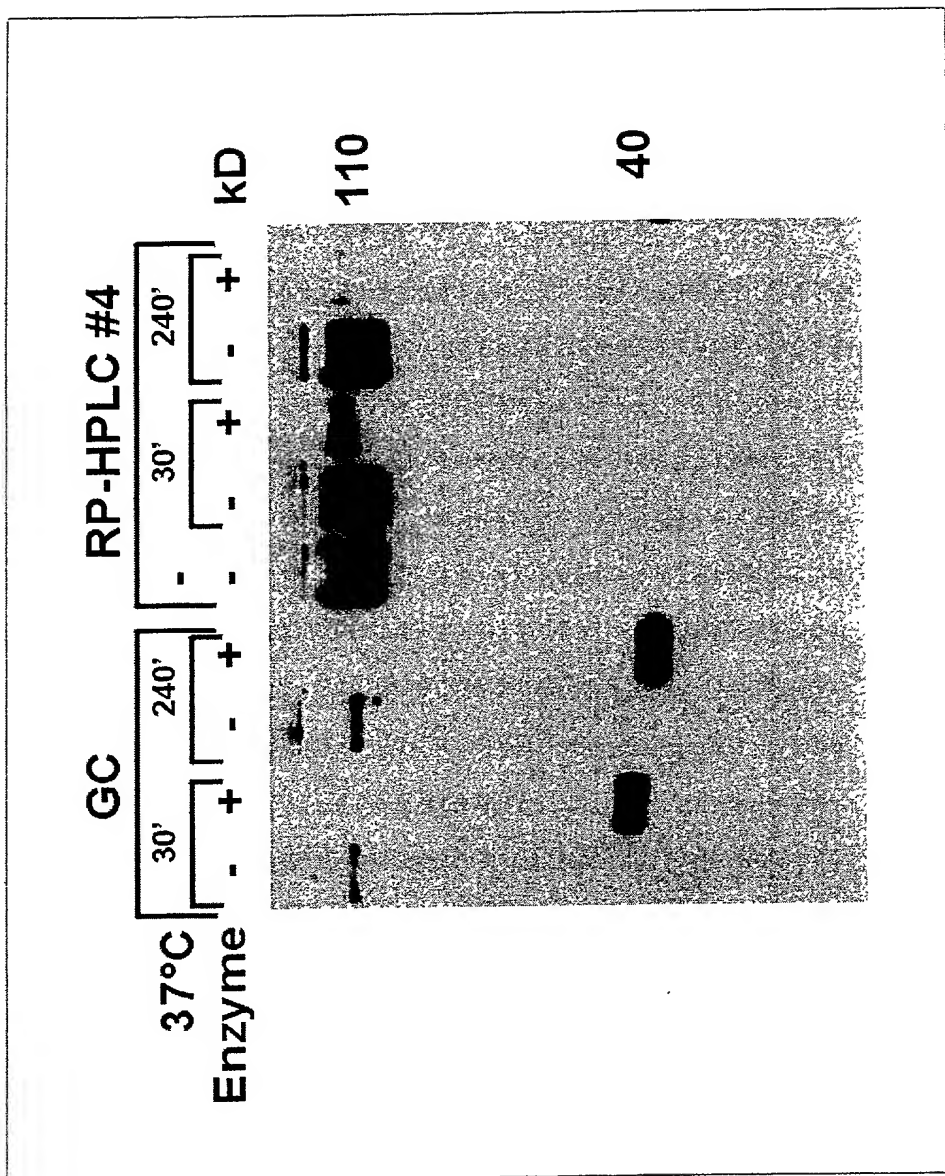
FIG. 21

**Y1-Ligand from KG1 membranes following
Immuno-Precipitation with Y1:
Purification on RP-HPLC**



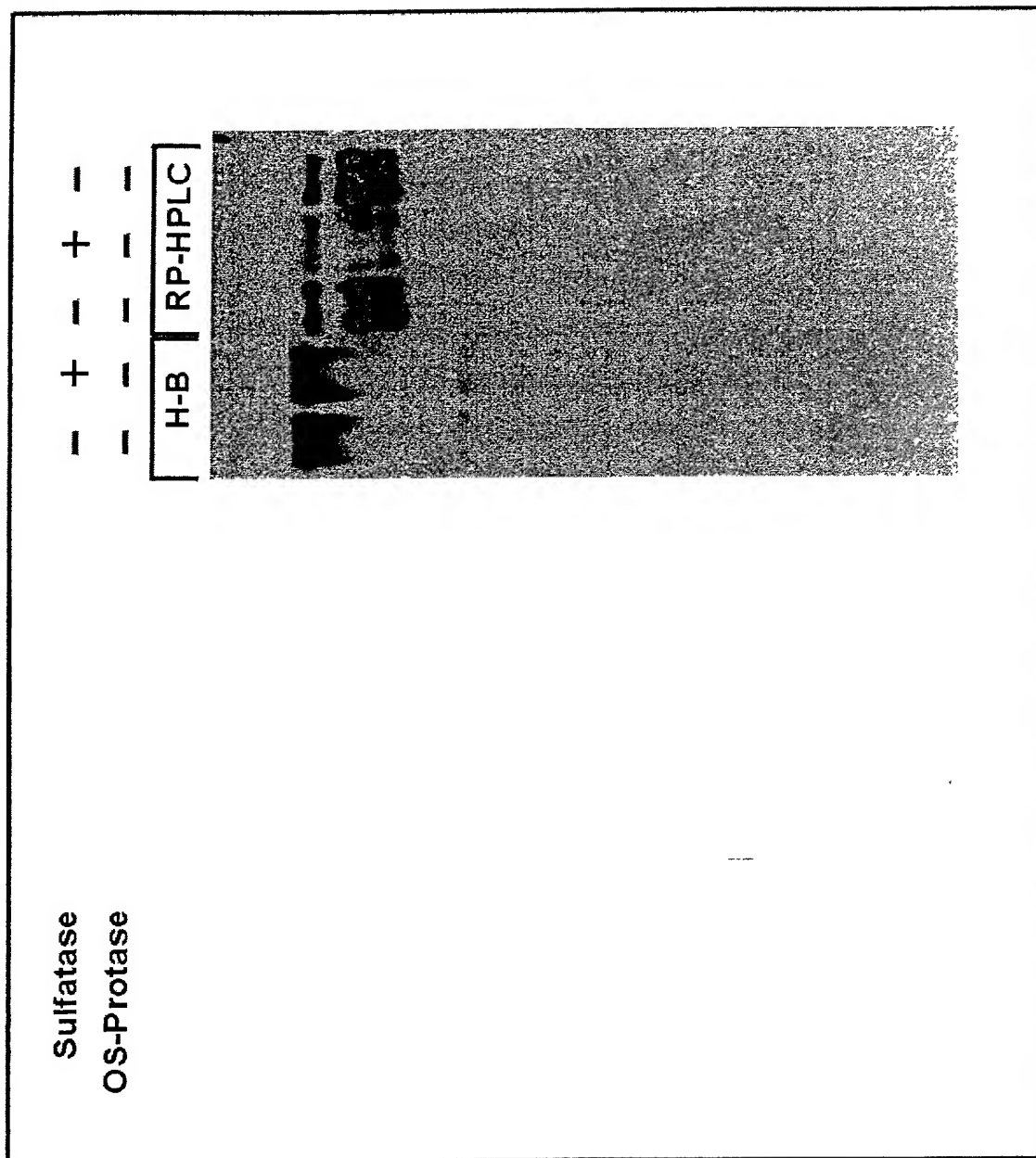
Effect of O-Sialo-Glycoprotein Endopeptidase on Y1 Binding

FIG. 22



Effect of Aryl-Sulfatase on Binding of Y1: RP-HPLC(KG1) & H-B(Heparin-BSA)

FIG. 23



Specificity of Y1 Binding: Analysis by Immune Precipitation with Y1 and anti-PSGL-1

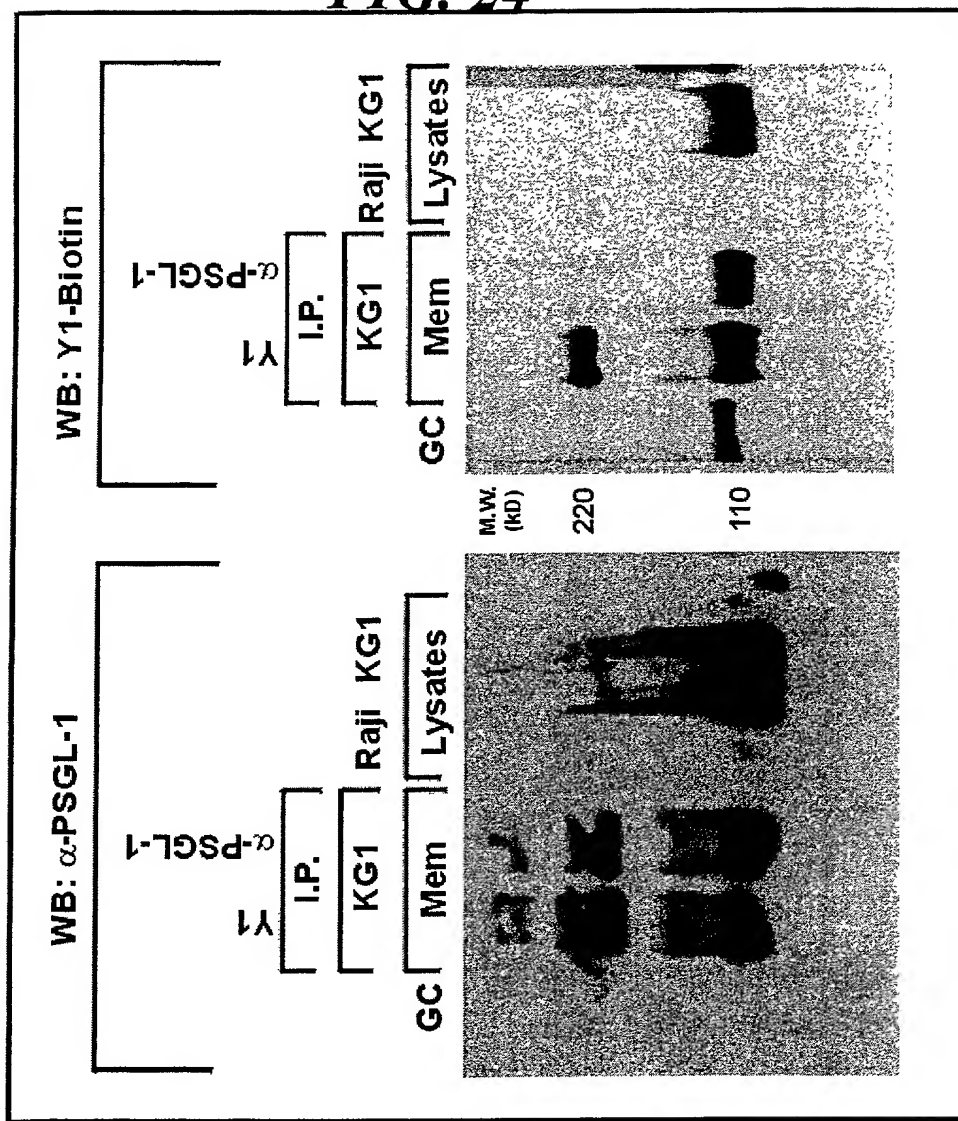
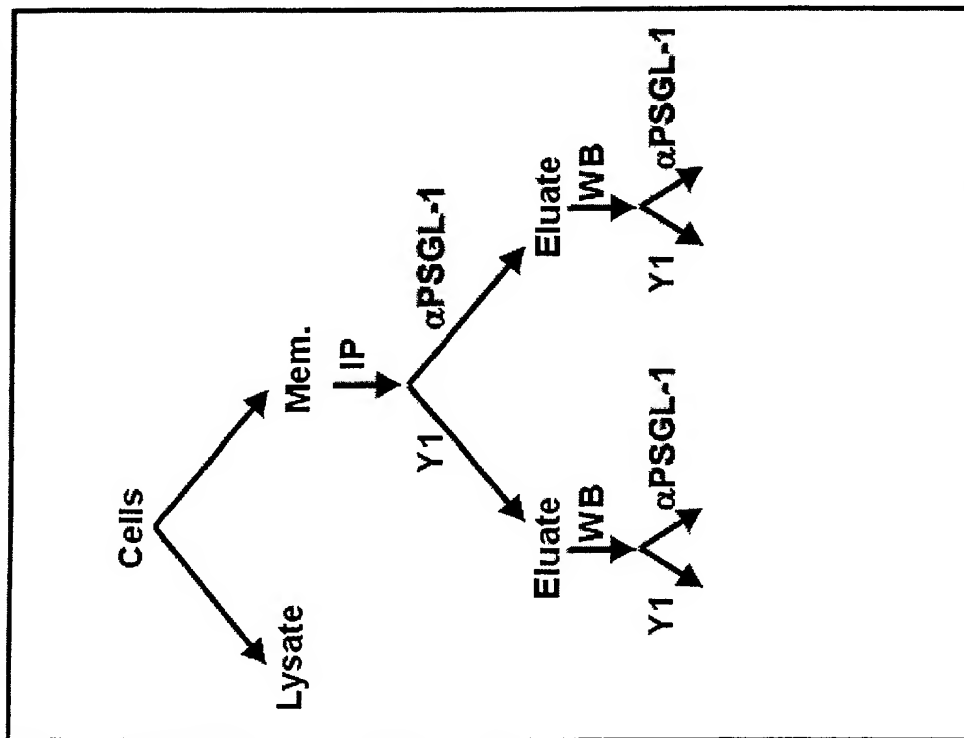
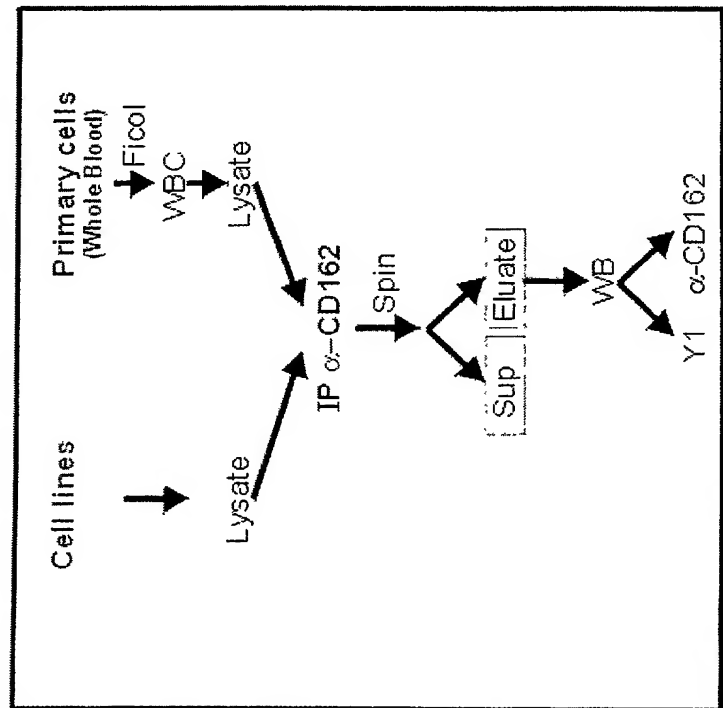


FIG. 24

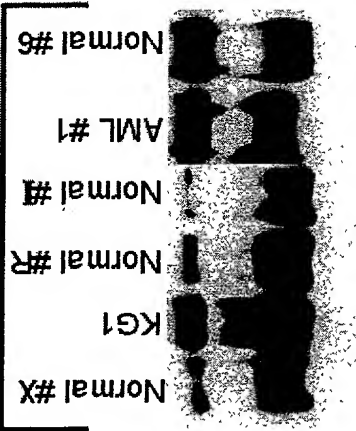
FIG. 25

α -CD162 and Y1:
Comparison between cells
from AML patient and normal
blood



I

WB: α PSGL-1

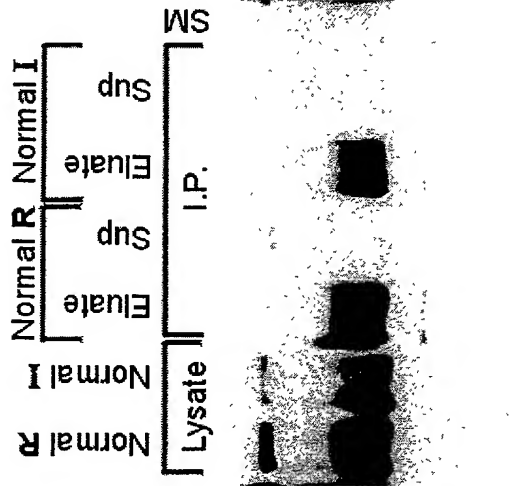


WB: Y1-B



II

WB: α PSGL-1



WB: Y1-B

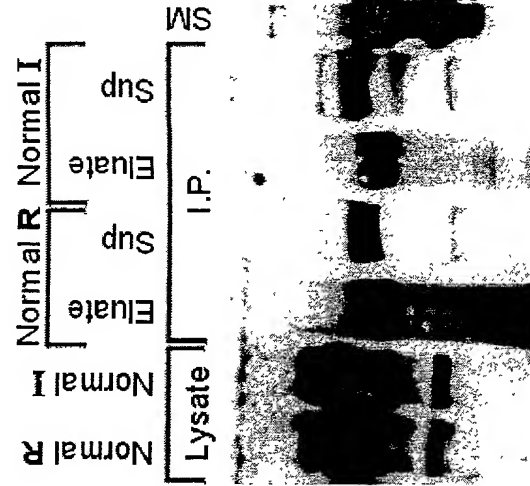
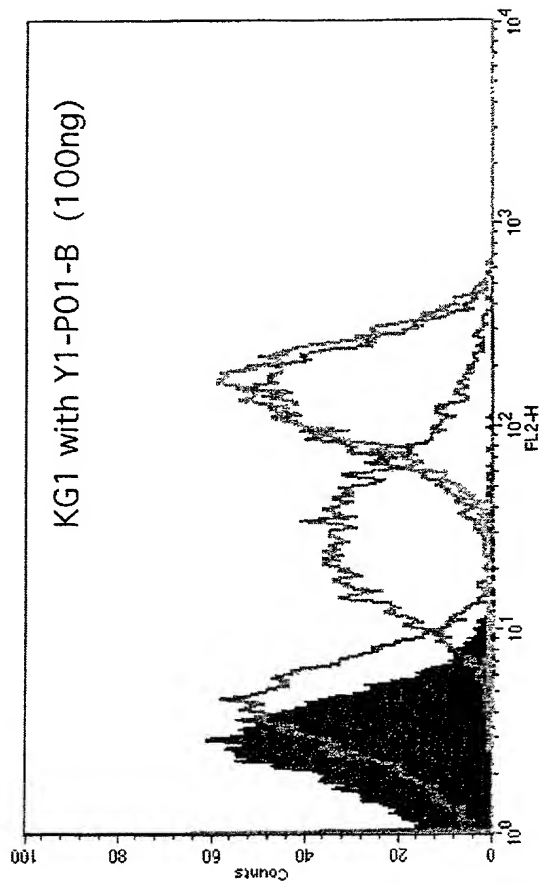
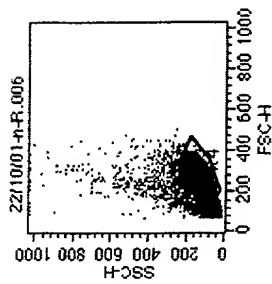
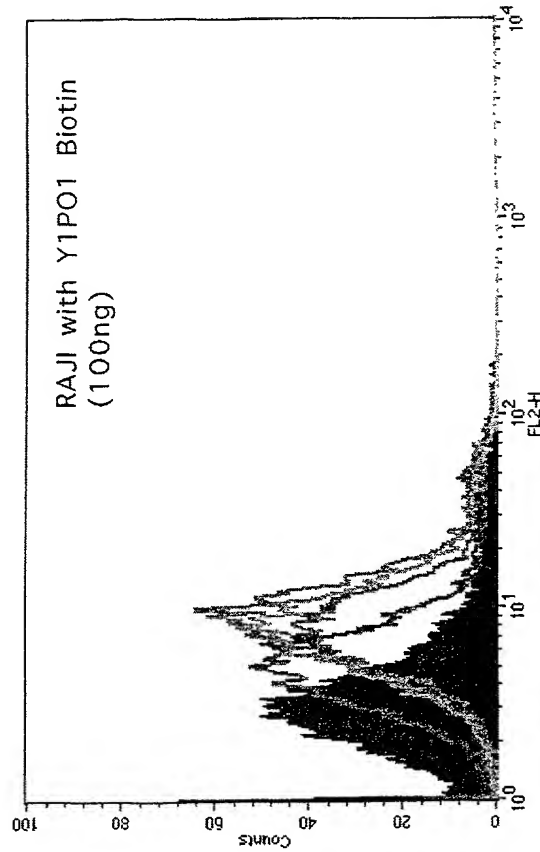
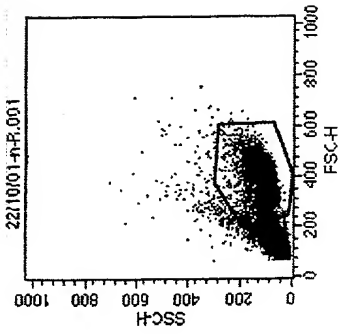


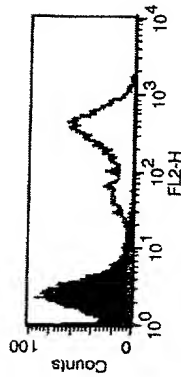
FIG. 26



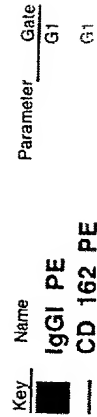
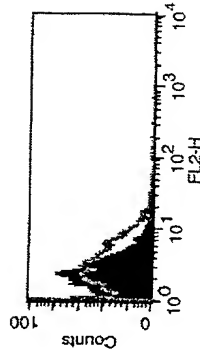
Key	Name	Parameter	Gate
	22/10/01-n-R.006	N01-B	
	22/10/01-n-R.006	P01-B	
	22/10/01-n-R.006	+KPL1	
	22/10/01-n-R.006	+PL1	
	22/10/01-n-R.006	+PL2	

Specificity of Y1 Binding: Analysis by FACS

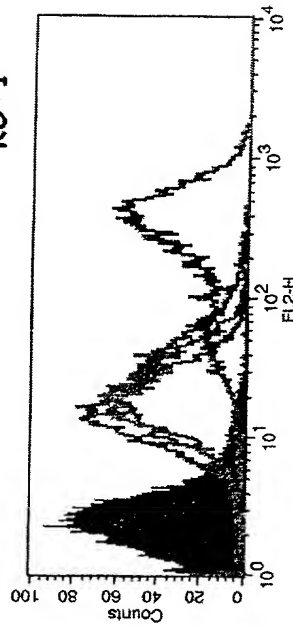
KG-1



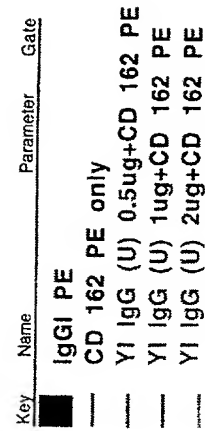
Raji



KG-1



CD 162 PE



- Binding of
 α PSGL1
(α CD162/KPL1);
competition
with Y1-IgG

FIG. 27

Specificity of Y1 Binding: Analysis by FACS

- Binding of Y1-IgG; competition with α PSGL-1 (CD162 /KPL1)

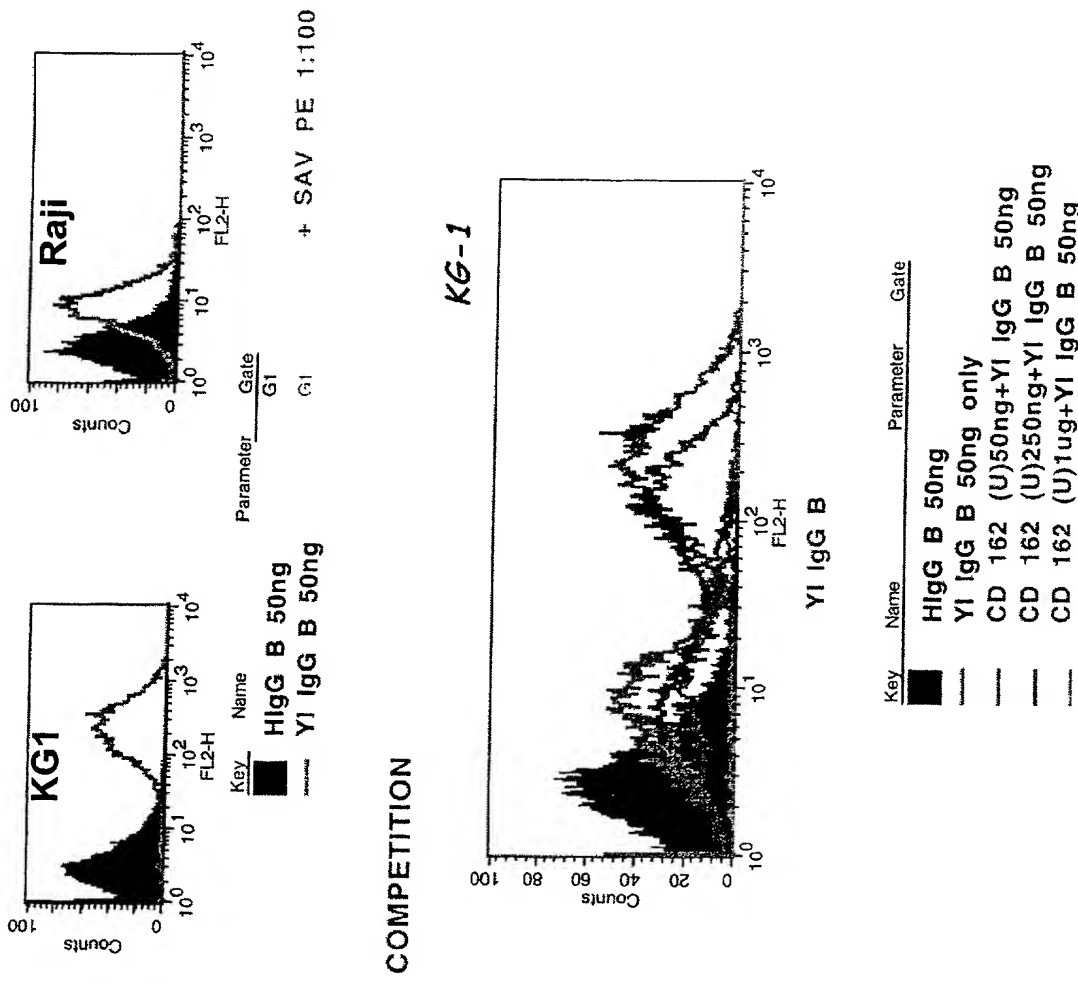


FIG. 28

FIG. 29

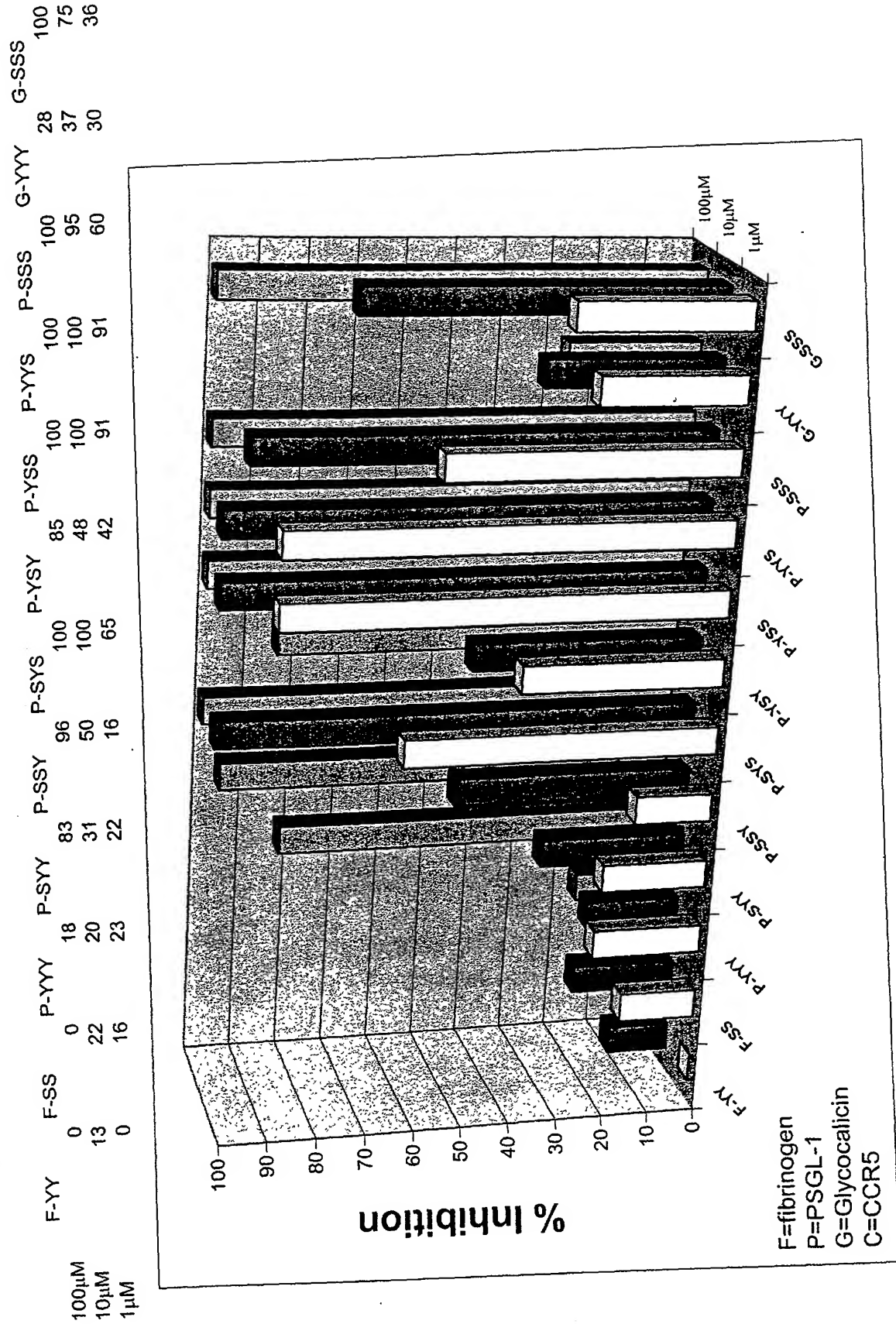


FIG. 30

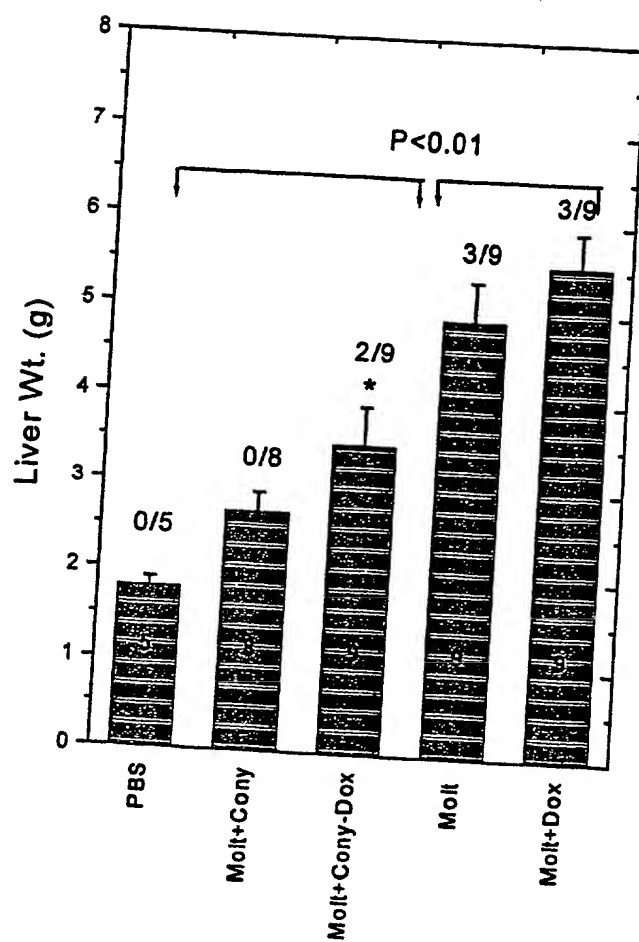


FIG. 31

*Ns were: 9 for DOX, 8 for CONY1, 7 for Y1-DOX 6 for MOLT and 5 for PBS.

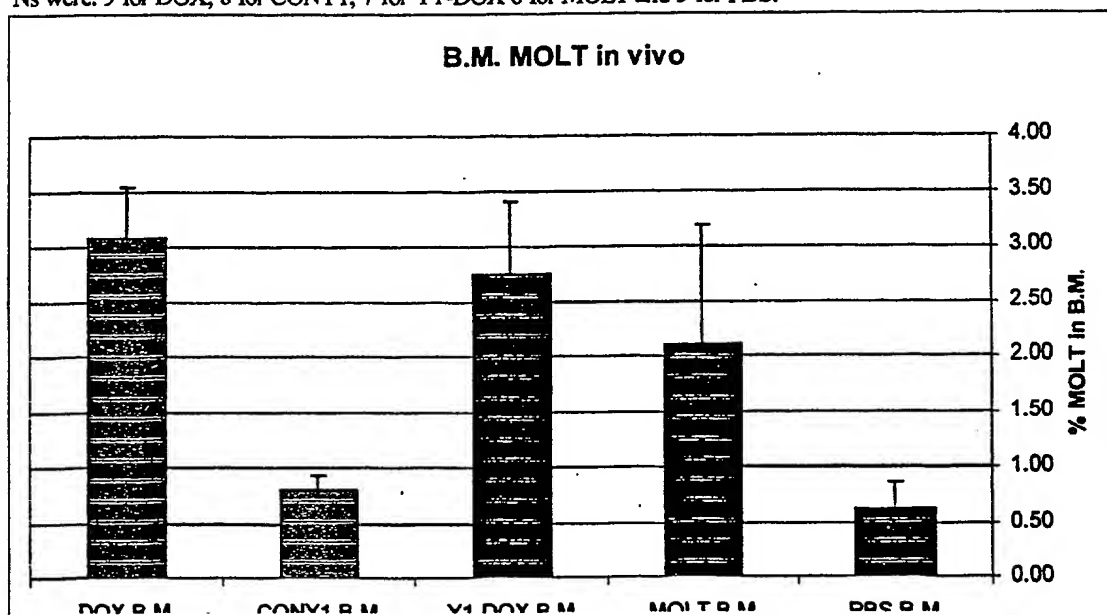
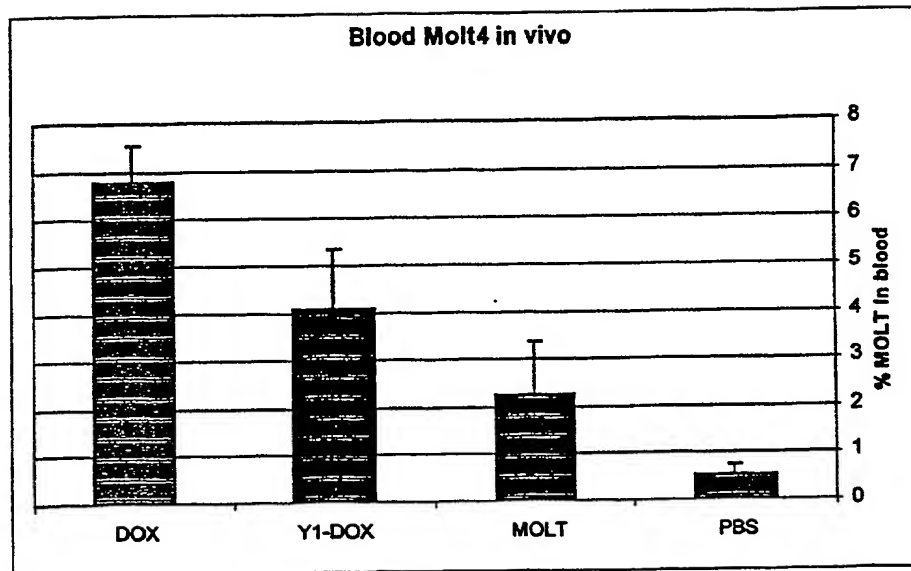


FIG. 32



****Ns were: 4 for DOX, 2 for Y1-DOX, 3 for MOLT and 3 for PBS.**

FIG. 33

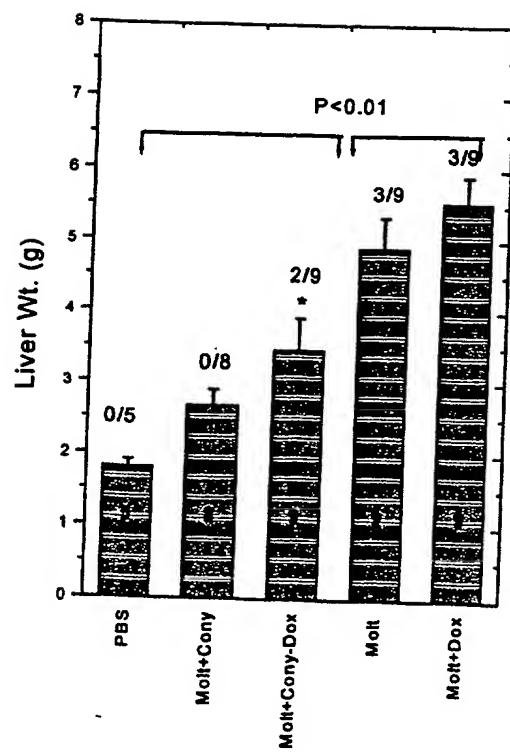


FIG. 34

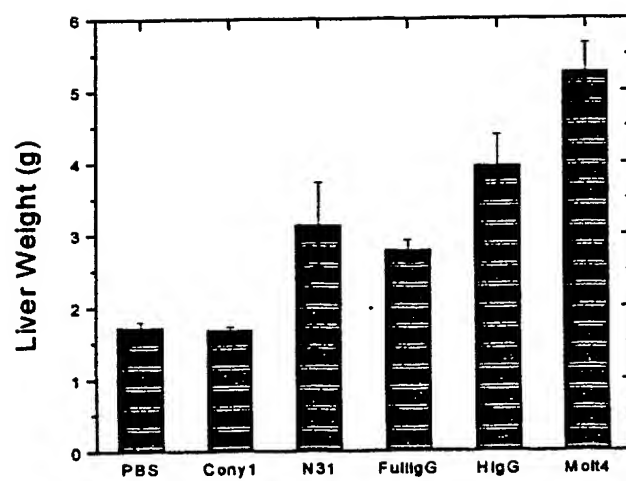


FIG. 35

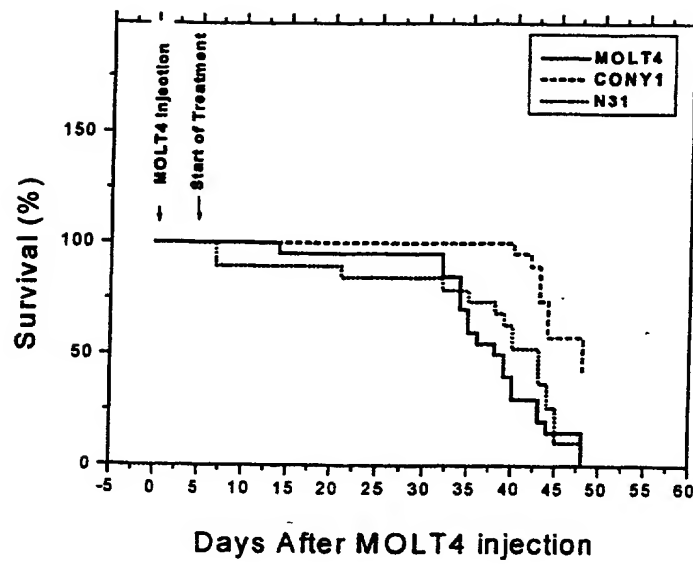


FIG. 36

***Ns were: 8 for PBS, 9 for KG1, 8 for CONY1, 11 for CONY1-DOX, 9 for DOX, 8 for 181 in vitro, 9 for Y1 in vitro and 9 for Mylotarg.

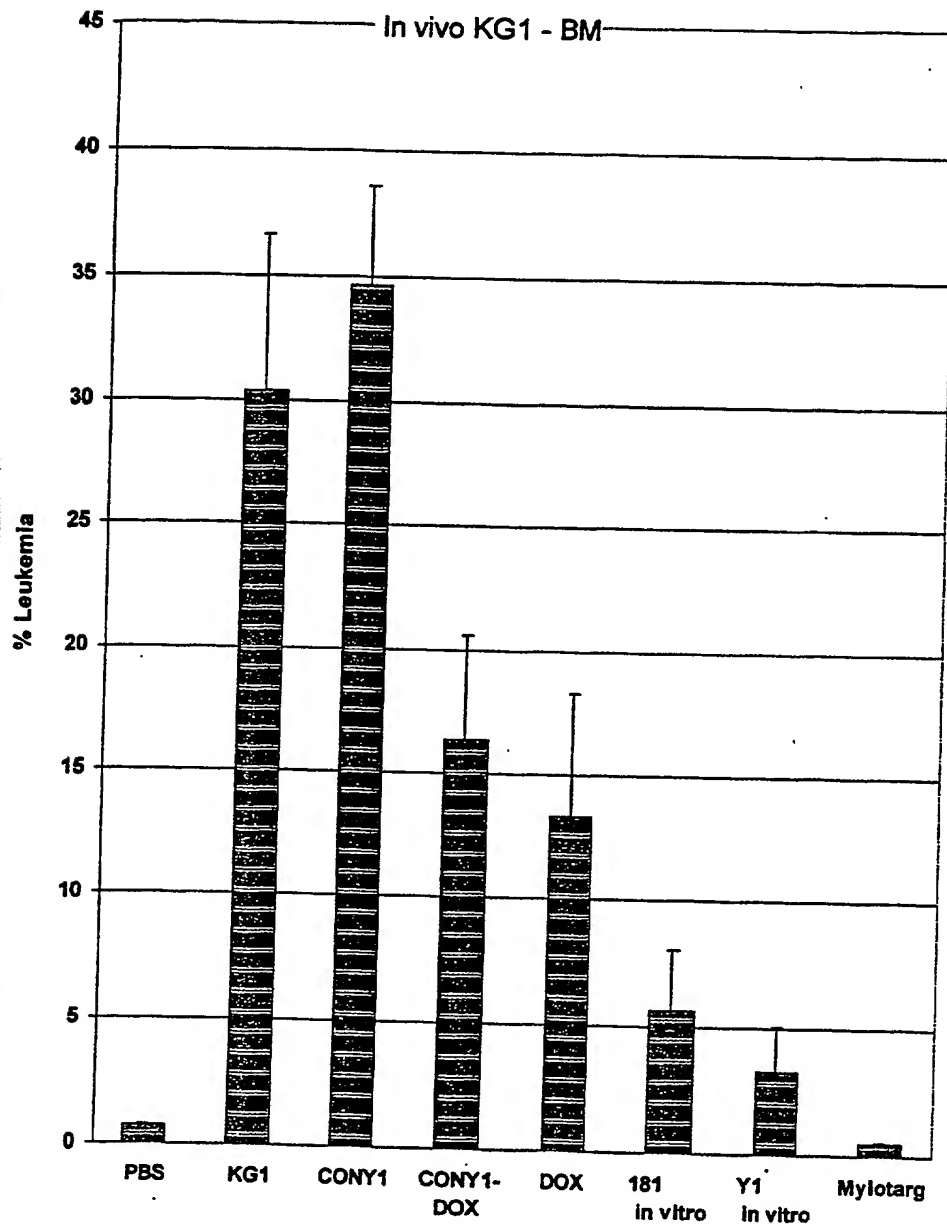
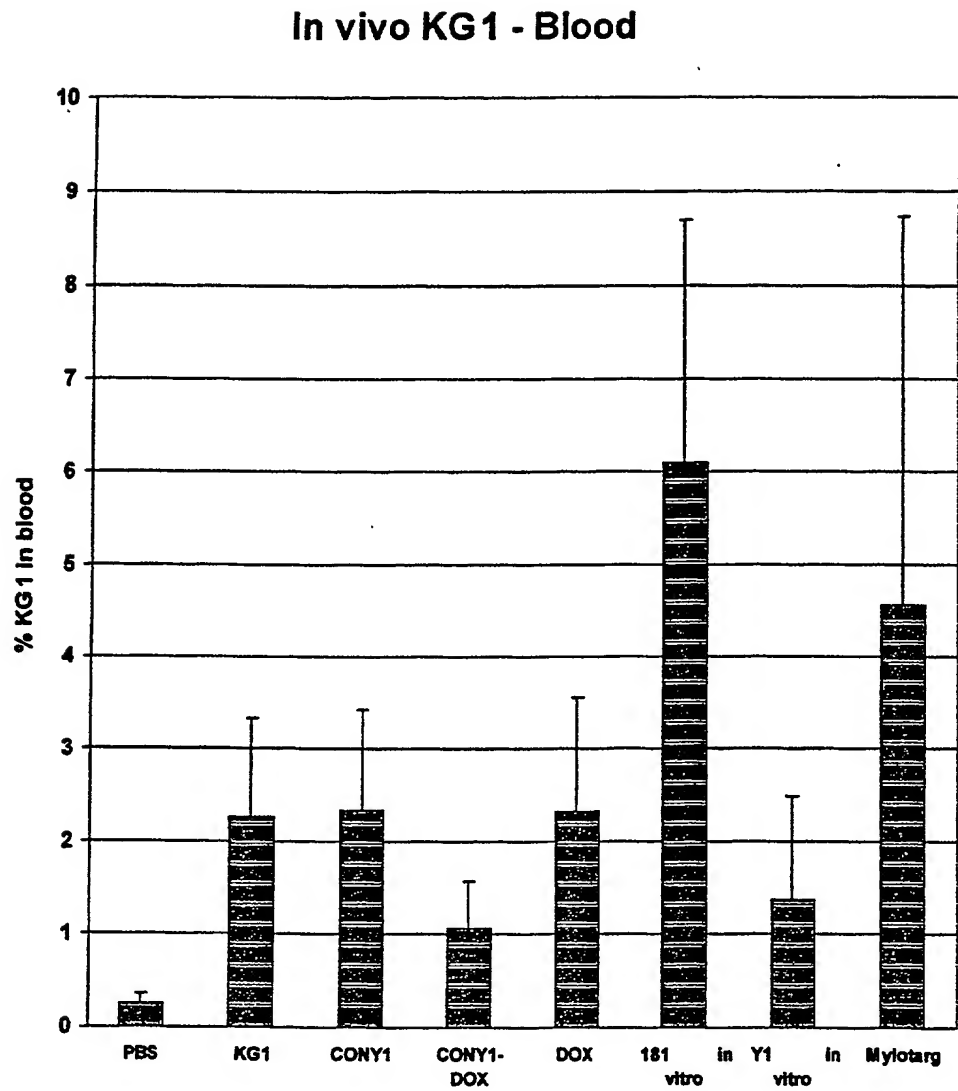


FIG. 37



****Ns were: 8 for PBS, 9 for KG1, 8 for CONY1, 9 for CONY1-DOX, 11 for DOX (including one mice injected with 5mg/kg DOX), 7 for 181 in vitro, 8 for Y1 in vitro and 7 for Mylotarg.

FIG. 38

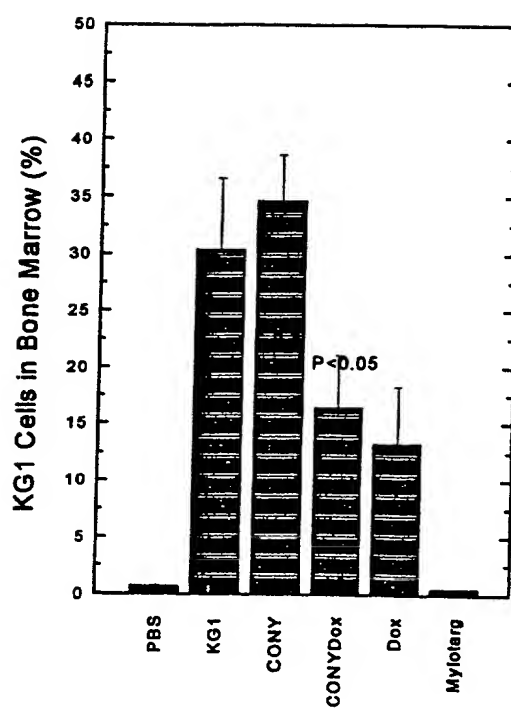
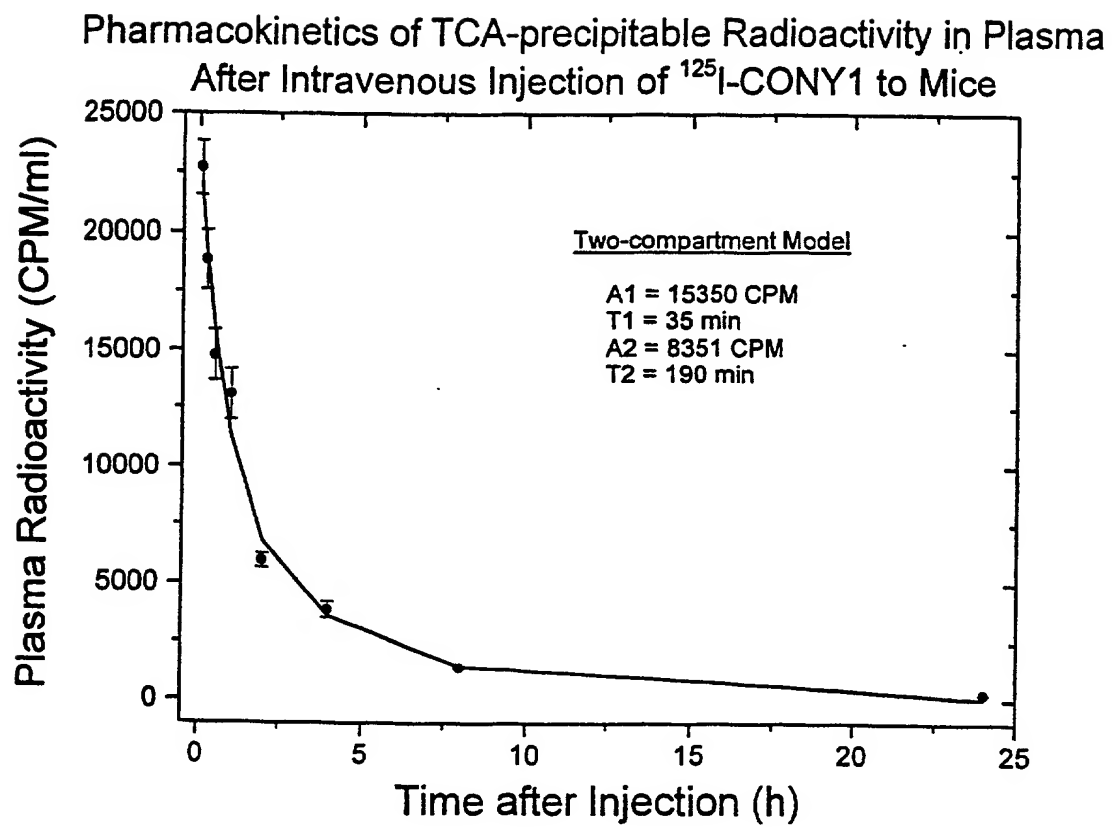


FIG. 39



Case	Age	Sex	Occupation	Duration of Illness	Site of Lesion	Microscopic Findings	Diagnosis
1	45	M	Farmer	10 years	Right lung	Granulomatous inflammation with caseation	Tuberculosis
2	35	F	Teacher	5 years	Left lung	Granulomatous inflammation with caseation	Tuberculosis
3	55	M	Engineer	15 years	Right lung	Granulomatous inflammation with caseation	Tuberculosis
4	65	F	Homemaker	20 years	Left lung	Granulomatous inflammation with caseation	Tuberculosis
5	40	M	Student	3 years	Right lung	Granulomatous inflammation with caseation	Tuberculosis
6	50	F	Teacher	10 years	Left lung	Granulomatous inflammation with caseation	Tuberculosis
7	60	M	Engineer	15 years	Right lung	Granulomatous inflammation with caseation	Tuberculosis
8	70	F	Homemaker	25 years	Left lung	Granulomatous inflammation with caseation	Tuberculosis
9	42	M	Student	4 years	Right lung	Granulomatous inflammation with caseation	Tuberculosis
10	52	F	Teacher	12 years	Left lung	Granulomatous inflammation with caseation	Tuberculosis

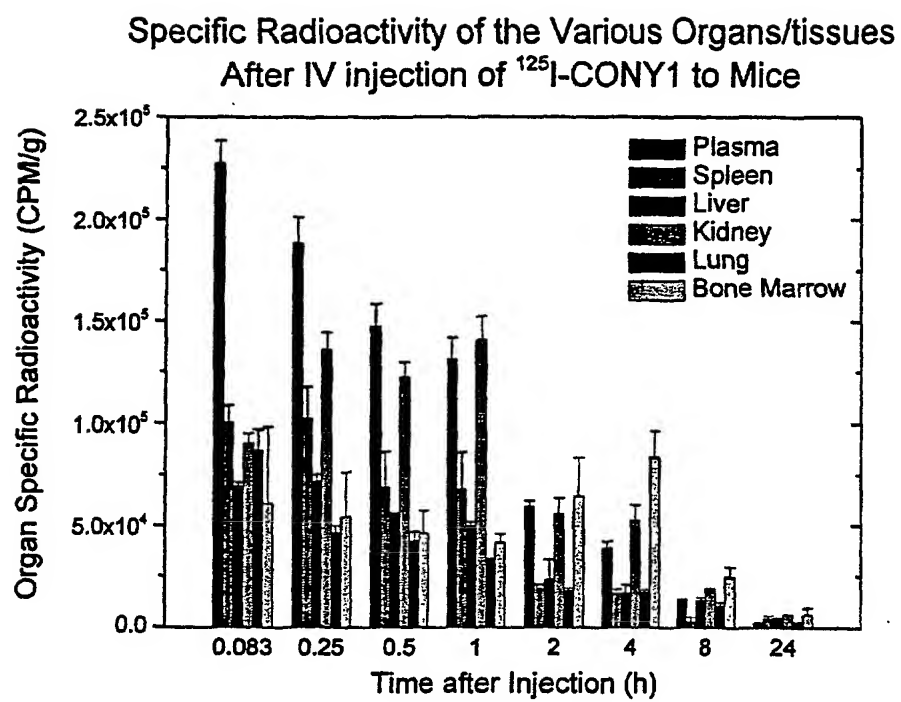


FIG. 41

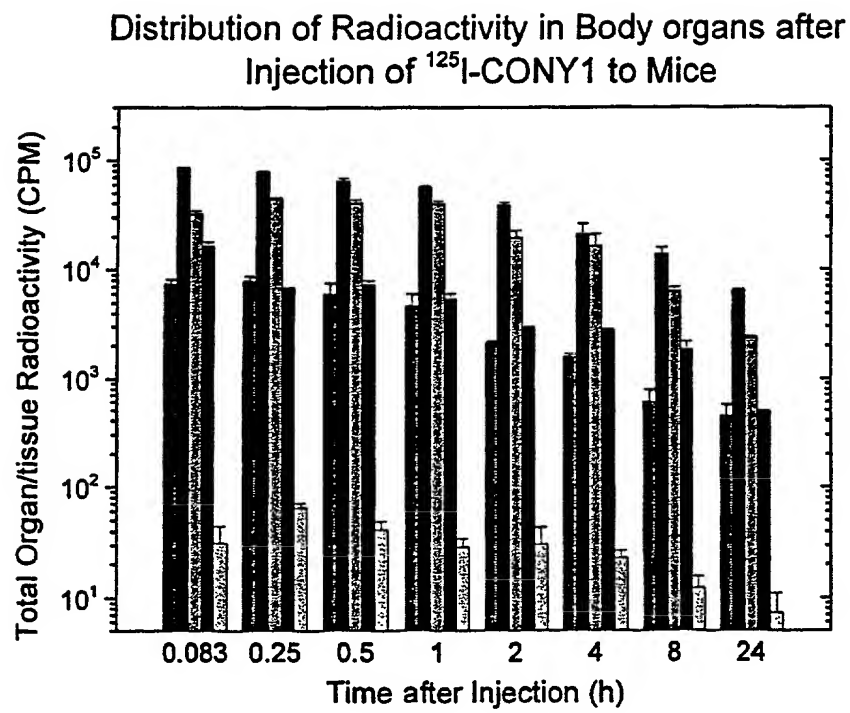


FIG. 42

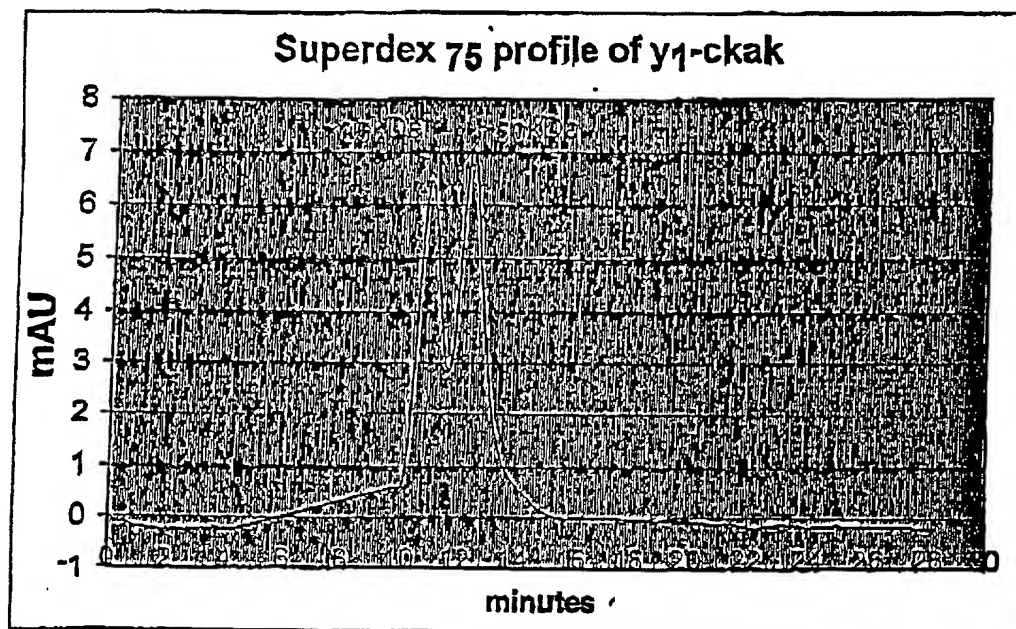


FIG. 43

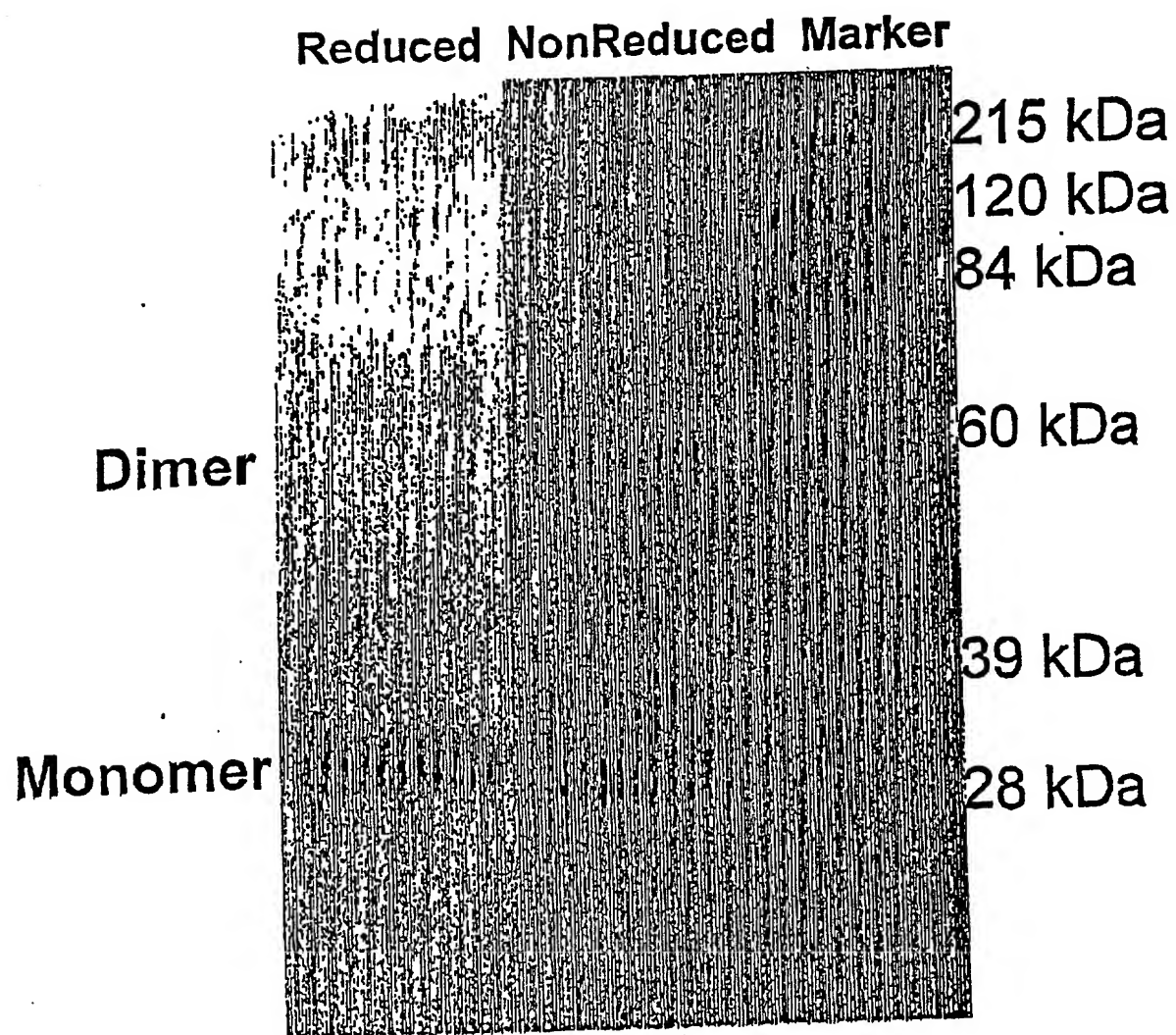


FIG. 44

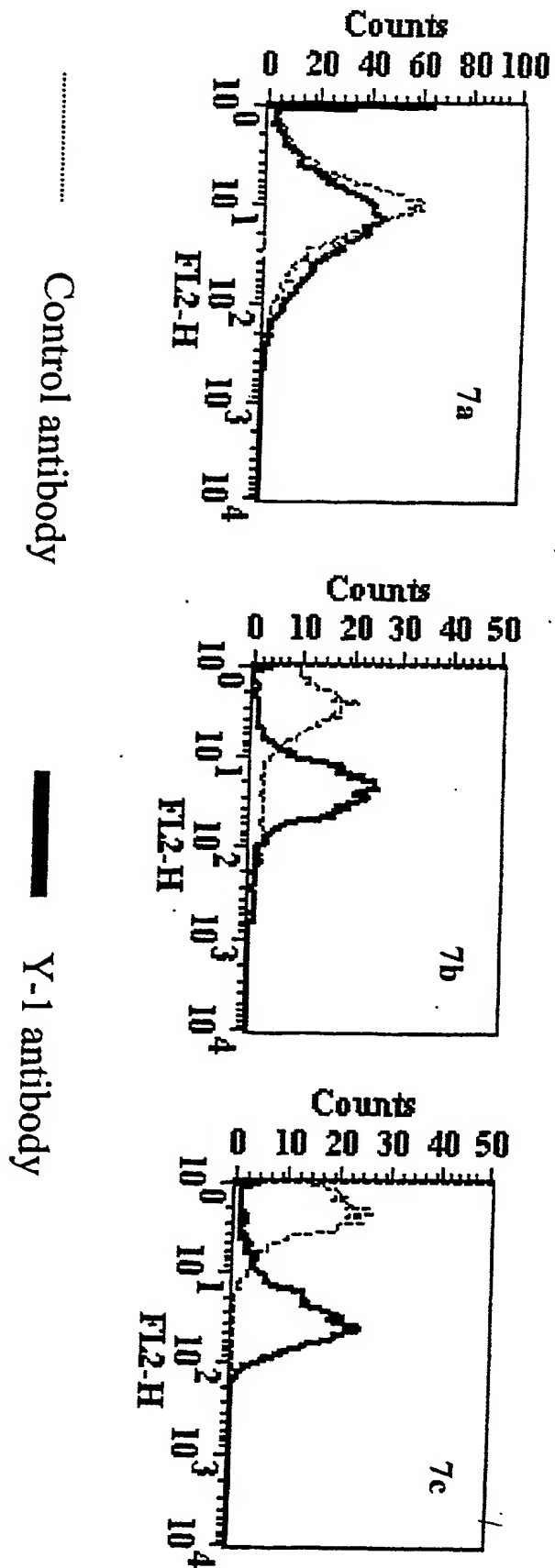


FIG. 45

Epitopes of anti-GPIb α antibodies

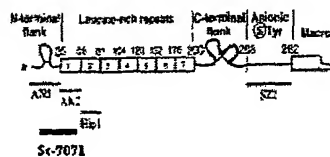
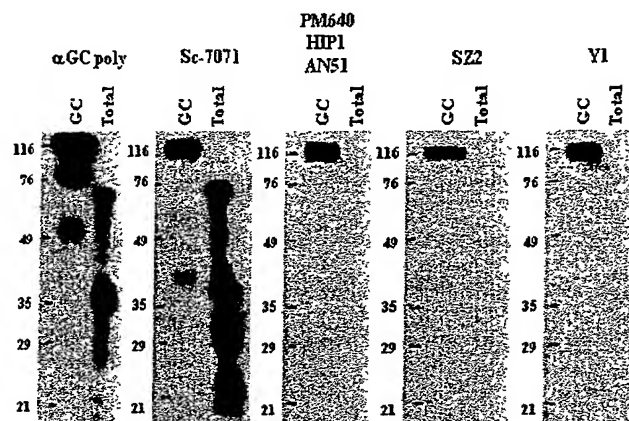


FIG. 46

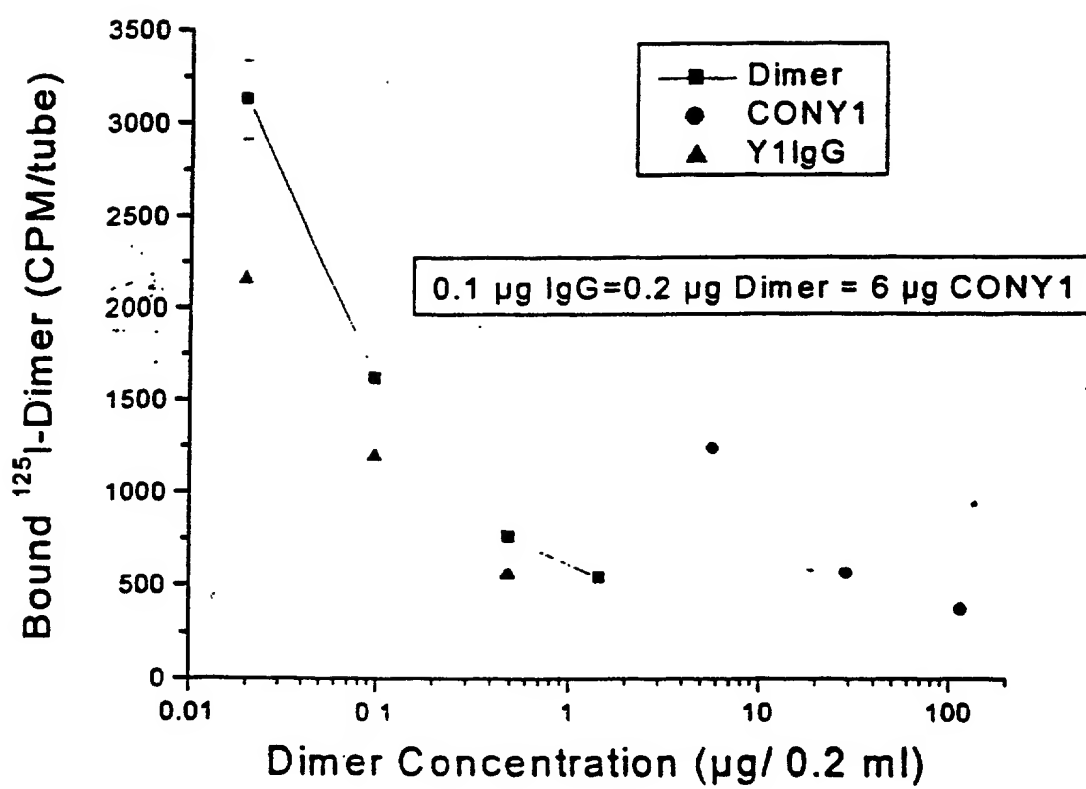


FIG. 47

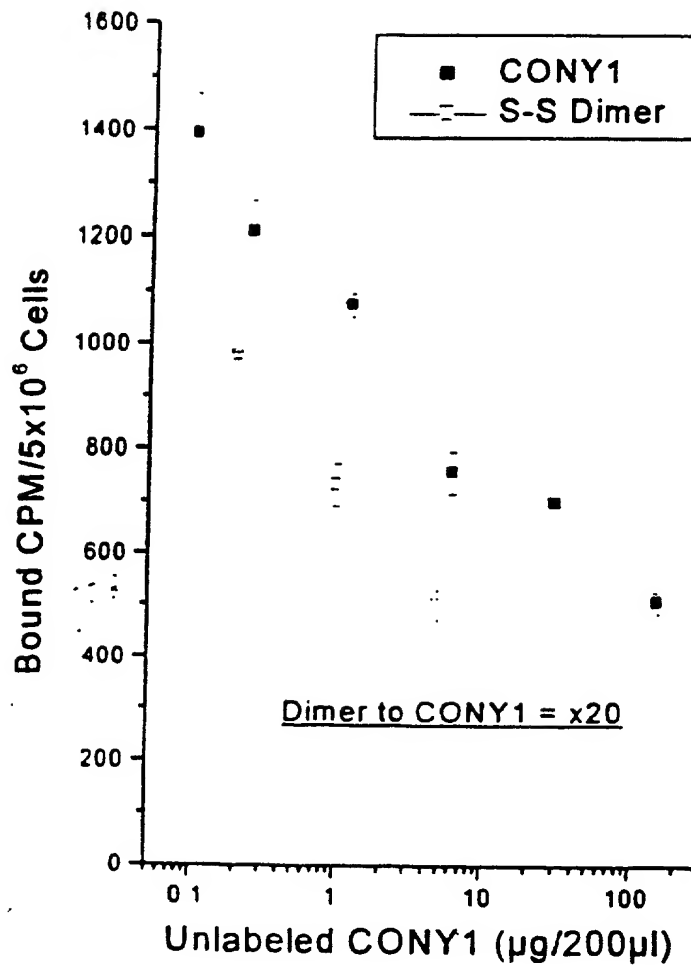


FIG. 48A: The ORF and Amino Acid Sequence of Y1-HC

SEQ ID NO: 205 (nucleic acid sequence); SEQ ID NO: 206 (amino acid sequence)

1	ATGGCCTGGGCTCTGCTGCTCCTOACCCTCCTCACTCAGGACACAGGGTCTGGGCCGAT
1	<u>M A W A L L L L T L L T Q D T G S W A D</u>
61	ATCCAGCTGCTGGAGTCTGGGGGAGGTGTGGTACGGCCTGGGGGGTCCCTGAGACTCTCC
21	I Q L V E S G G G V V R P G G S L R L S
121	TGTGCAGCCTCTGGATTACCTTTGATGATTATGGCATGAGCTGGGTCCGCCAAGCTCCA
41	C A A S G F T F D D Y G M S W V R Q A P
181	GGGAAGGGGCTGGAGTGGGTCTCTGGTATTAATTGGAATGGTGGTAGCACAGGTTATGCA
61	G K G L E W V S G I N W N G G S T G Y A
241	GACTCTGTGAAGGGCCGATTACCATCTCTAGAGACAACGCCAAGAACTCCCTGTATCTG
81	D S V K G R F T I S R D N A K N S L Y L
301	CAAATGAACAGTCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCAAGAATGAGGGCT
101	Q M N S L R A E D T A V Y Y C A R M R A
361	CCTGTGATTTGGGGCCAAGGTACCCTGGTCACCGTCTCGAGTGCTTCCACCAAGGGCCCA
121	P V I W G Q G T L V T V S S A S T K G P
421	TCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGC
141	S V F P L A P S S K S T S G G T A A L G
481	TGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAAGTCAAGGCGCCCTG
161	C L V K D Y F P E P V T V S W N S G A L
541	ACCAGCGGCGTGACACCTTCCCGGCTGTCTACAGTCCTCAGGACTCTACTCCCTCAGC
181	T S G V H T F P A V L Q S S G L Y S L S
601	AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAAT
201	S V V T V P S S S L G T Q T Y I C N V N
661	CACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAAC
221	H K P S N T K V D K R V E P K S C D K T
721	CACACATGCCCACCGTGCCAGCACCTGAACTCCTGGGGGGACTGTGAGTCTTCOTCTTC
241	H T C P P C P A P E L L G G P S V F L F
781	CCCCCAAACCCAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTG
261	P P K P K D T L M I S R T P E V T C V V
841	GTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAG
281	V D V S H E D P E V K F N W Y V D G V E
901	GTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
301	V H N A K T K P R E E Q Y N S T Y R V V
961	AGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTC
321	S V L T V L H Q D W L N G K E Y K C K V
1021	TCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCCC
341	S N K A L P A P I E K T I S K A K G Q P
1081	OGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTC
361	R E P Q V Y T L P P S R E E M T K N Q V
1141	AGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGC
381	S L T C L V K G F Y P S D I A V E W E S
1201	AATGGGCAGCCGGAGAACAACCTACAAGACCACGTCTCCCGTGCTGGACTCCGACGGCTCC
401	N G Q P E N N Y K T T S P V L D S D G S
1261	TTCTTCCTCTATAGCAAGCTCACCGTGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTC
421	F F L Y S K L T V D K S R W Q Q G N V F
1321	TCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTG
441	S C S V M H E A L H N H Y T Q K S L S L
1381	TCTCTGGGTAAATGA
461	S L G K *

FIG. 48B: The ORF and Amino Acid Sequence of Y1-LC

SEQ ID NO: 207 (nucleic acid sequence); SEQ ID NO: 208 (amino acid sequence)

1	ATGGCCTGGGCTCTGCTGCTCCTCACCCCTCCTCACTCAGGACACAGGGTCCTGGGCCGAT
1	<u>M A W A L L L L T L L T Q D T G S W A D</u>
61	GCAGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCACA
21	A E L T Q D P A V S V A L G Q T V R I T
1212	TGCCAAGGAGACAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACAG
41	C Q G D S L R S Y Y A S W Y Q Q K P G Q
181	GCCCCTGTACTTGTCTATCTATGGTAAAAACAACCGGCCCTCAGGGATCCCAGACCGATTTC
161	A P V L V I Y G K N N R P S G I P D R F
241	TCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGAT
81	S G S S S G N T A S L T I T G A Q A E D
301	GAGGCTGACTATTACTGTAACTCCCGGGACAGCAGTGGTAACCATGTGGTATTCGGCGGA
101	E A D Y Y C N S R D S S G N H V V F G G
361	GGGACCAAGCTGACCGTCCTAGGTCTAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCG
121	G T K L T V L G Q P K A A P S V T L F P
421	CCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTC
141	P S S E E L Q A N K A T L V C L I S D F
481	TACCCGGGAGCCGTGACAGTGGCCTGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTG
161	Y P G A V T V A W K A D S S P V K A G V
541	GAGACCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTACCTGAGC
181	E T T T P S K Q S N N K Y A A S S Y L S
601	CTGACGCCTGAGCAGTGGGAAGTCCCACAAAAGCTACAGCTGCCAGGTACGCATGAAGGG
201	L T P E Q W K S H K S Y S C Q V T H E G
661	AGCACCGTGGAGAAGACAGTGGCCCCCTACAGAATGTTTCATGA
221	S T V E K T V A P T E C S *

FIG. 49

	1	11	21	31	41	51	
1	EVOLVESGGG	LVQPGGSLRL	SCAASGFTFS	SYAMSWVRQA	PGKGLEWVSA	ISGSGGSTYY	60
61	ADSVKGRETI	SRDNSKNTLY	LOMNSLRAED	TAVYYCARYA	KTLMRQYSLW	GQGTLVTVSR	120
121	GGGSGGGGS	GGGGSSELTQ	DPAVSVALGQ	TVRITCQGDS	LASYIASWYQ	QKPGQAPVLV	180
181	IYGKNNRPSG	IPDRFSGSSS	GNTASLTITG	AQAEDEADYY	CNSRDSSGNH	VVFGGGTKLT	240
241	VLGAAAEQKL	ISEEDLNGAA					

FIG. 50

		10	20	30	40	50	60
1	3	ATTATTACTc gCGGCCcAGC CgGCCcAGC CGAGGTGCAG CTGGTGGAGT CTGGGGGAGG					
		L L L A A Q P A M A E V Q L V E S G G G					
		70	80	90	100	110	120
1	3	CTGGGTACAG CCTGGGGGGT CCTGAGACT CTCCTGTGCA GCCTCTGGAT TCACCTTTAG					
		L V Q P G G S L R L S C A A S G F T F S					
		130	140	150	160	170	180
1	3	CAGCTATGCC ATGAGCTGGG TCCGCCAGGC TCCAGGGAAG GGGCTGGAGT GGGTCTCAGC					
		S Y A M S W V R Q A P G K G L E W V S A					
		190	200	210	220	230	240
1	3	TATTAGTGGT AGTGGTGGTA GCACATACTA CGCAGACTCC GTGAAGGGCC GGTTCACCAT					
		I S G S G G S T Y Y A D S V K G R F T I					
		250	260	270	280	290	300
1	3	CTCCAGAGAC AATTCCAAGA ACACGCTGTA TCTGCAAATG AACAGCCTGA GAGCCGAGGA					
		S R D N S K N T L Y L Q M N S L R A E D					
		310	320	330	340	350	360
1	3	CACGGCCGTG TATTACTGTG CAAGACCGG TCAGATTAATTAAGCGTAAT GGGGCCAAGG					
		T A V Y Y C A R T G Q S I K R S W G Q G					
		370	380	390	400	410	420
1	3	TACCCTGGTC ACCGTGTGCA GAGGTGGAGG CGGTTCAGGC GGAGGTGGCT CTGGCGGTGG					
		T L V T V S R G G G S G G G G S G G G					
		430	440	450	460	470	480
1	3	CGGATCGTCT GAgCTGACTC AGGACCCTGC TGTGTCTGTG GcCTTGGGAC AgACAGTCAG					
		G S S E L T Q D P A V S V A L G Q T V R					
		490	500	510	520	530	540
1	3	GATcACATGC CAAGGagACA GCCTCAGAAG CTATTATGCA AGCTGGTACC AGCAGAAGCC					
		I T C Q G D S L R S Y Y A S W Y Q Q K P					
		550	560	570	580	590	600
1	3	AGGACAGGCC CCTGTACTTG TCATCTATGG TAAAAACAAC CGGCCCTCAG GGATCCCAGA					
		G Q A P V L V I Y G K N N R P S G I P D					
		610	620	630	640	650	660
1	3	CCGATTCTCT GGCTCCAGCT CAGGAAACAC AGCTTCCTTG ACCATCACTG GGGCTCAGGC					
		R F S G S S S G N T A S L T I T G A Q A					
		670	680	690	700	710	720
1	3	GGAAGATGAG GCTGACTATT ACTGTAACTC CCGGGACAGC AGTGGTAACC ATGTGGTATT					
		E D E A D Y Y C N S R D S S G N H V V F					
		730	740	750	760	770	780
1	3	CGGCGGAGGG ACCAAGCTGA CCGTCCTAGG TGCGGCCGCA GAACAAAAAC TCATCTCAGA					
		G G G T K L T V L G A A A E Q K L I S E					
		790	800	810	820	830	840
1	3	AGAgGatCTG AatGGGGCCG CAAGGAACTG TtGAATTTT TAAGTTAAcC T					
		E D L N G A A * N C * I F * V N					

Y16 SEQ ID NO: 210

FIG. 51

Sequence of Y1-Biotag (SEQ ID NO: 211)

1 MEVQLVESGG GVVRPGGSLR LSCAASGFTF DDYGMSWVRQ
41 APGKGLEWVS GINWNGGSTG YADSVKGRFT ISRDNAKNSL
81 YLQMNSLRAE DTA VYYCARM RAPVIWGQGT LTVSRGGGG
121 SGGGGSGGGG SSEL TQDPAV SVALGQTVRI TCQGDSLRSY
161 YASWYQQKPG QAPVLVIYGK NNRPSGIPDR FSGSSSGNTA
201 SLTITGAQAE DEADYYCNSR DSSGNNVVFG GGTKLTVLGG
241 GGLNDIFEAQ KIEWHE

FIG. 52

Y1-cys-kak scFv (SEQ ID NO. 212)

1 MEVQLVESGG GVVVRPGGSLR LSCAASGFTF DDYGMSWVRQ
APGKGLEWVS GINWNGGSTG 60

61 YADSVKGRFT ISRDNAKNSL YLQMNSLRAE DTAVYYCARM
RAPVIWGQGT LVTVSRGGGG 120

121 SGGGGSGGGG SSELTDPAV SVALGQTVRI TCQGDSLRSY
YASWYQQKPG QAPVLVIYGK 180

181 NNRPSGIPDR FSGSSSGNTA SLTITGAQAE DEADYYCNSR
DSSGNHVVFG GGTKLTVLGG 240

241 GGCKAK